



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12Q 1/44, 1/48, 1/68, C12P 19/34,</b> <b>C12N 9/22, 15/00, C07H 21/02, 21/04</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/26401</b>  <b>(43) International Publication Date:</b> 11 May 2000 (11.05.00)
<b>(21) International Application Number:</b> PCT/US99/25251 <b>(22) International Filing Date:</b> 2 November 1999 (02.11.99)  <b>(30) Priority Data:</b> 60/106,925                      3 November 1998 (03.11.98)                      US 09/309,175                      10 May 1999 (10.05.99)                      US  <b>(71) Applicant:</b> THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE [US/US]; 720 Rutland Avenue, Baltimore, MD 21205 (US).  <b>(72) Inventors:</b> ISSA, Jean-Pierre; 12310 Longworth Lane, Hous- ton, TX 77024 (US). BAYLIN, Stephen; Apt. 419, Har- bor Hill Apartments, 301 Warren Avenue, Baltimore, MD 21230 (US). TOYOTA, Minoru; Apt. 102, 6804 Harrow- dale Road, Baltimore, MD 21209 (US).  <b>(74) Agent:</b> HAILE, Lisa, A.; Gray Cary Ware & Friedenrich LLP, Suite 1600, 4365 Executive Drive, San Diego, CA 92121-2189 (US).		<b>(81) Designated States:</b> AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
<b>(54) Title:</b> METHYLATED CpG ISLAND AMPLIFICATION (MCA)  <b>(57) Abstract</b>  <p>A method is provided for identifying a methylated CpG containing nucleic acid including contacting a nucleic acid with a methylation sensitive restriction endonuclease that cleaves unmethylated CpG sites, contacting the sample with an isoschizomer of the methylation sensitive restriction endonuclease, wherein the isoschizomer cleaves both methylated and unmethylated CpG sites. The method also includes adding an oligonucleotide under conditions and for a time to allow ligation of the oligonucleotide to nucleic acid cleaved by the restriction endonuclease, and amplifying the nucleic acid. A method is also provided for detecting an age associated disorder including contacting a nucleic acid with a methylation sensitive restriction endonuclease that cleaves unmethylated CpG sites, contacting the sample with an isoschizomer of the methylation sensitive restriction endonuclease, wherein the isoschizomer cleaves both methylated and unmethylated CpG sites. The method also includes adding an oligonucleotide under conditions and for a time to allow ligation of the oligonucleotide to nucleic acid cleaved by the restriction endonuclease, and amplifying the nucleic acid. The amplified digested nucleic acid is adhered to a membrane, and hybridized with a probe of interest. A kit useful for detection of a CpG containing nucleic acid is also provided.</p>		

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## METHYLATED CpG ISLAND AMPLIFICATION (MCA)

### STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

This invention was made with Government support under Grant No. CA43318 and CA54396, awarded by the National Cancer Institute and Grant No. CA43318, a Colon Cancer Spore Grant. The government may have certain rights in the invention.

### FIELD OF THE INVENTION

The present invention relates generally to regulation of gene expression and more specifically to a method of determining the DNA methylation status of CpG sites in a given locus.

### BACKGROUND OF THE INVENTION

DNA methylases transfer methyl groups from the universal methyl donor S-adenosyl methionine to specific sites on the DNA. Several biological functions have been attributed to the methylated bases in DNA. The most established biological function for methylated DNA is the protection of DNA from digestion by cognate restriction enzymes. The restriction modification phenomenon has, so far, been observed only in bacteria. Mammalian cells, however, possess a different methylase that exclusively methylates cytosine residues that are 5' neighbors of guanine (CpG). This modification of cytosine residues has important regulatory effects on gene expression, especially when involving CpG rich areas, known as CpG islands, located in the promoter regions of many genes.

Methylation has been shown by several lines of evidence to play a role in gene activity, cell differentiation, tumorigenesis, X-chromosome inactivation, genomic imprinting and other major biological processes (Razin, A., H., and Riggs, R.D. eds. in DNA Methylation Biochemistry and Biological Significance, Springer-Verlag, New York, 1984).

Methylation has been shown by several lines of evidence to play a role in gene activity, cell differentiation, tumorigenesis, X-chromosome inactivation, genomic imprinting and other major biological processes (Razin, A. H. and Riggs, R.D. eds. in DNA Methylation Biochemistry and Biological Significance, Springer-Verlag, New York, 1984). In eukaryotic cells, methylation of cytosine residues that are immediately 5' to a guanosine, occurs predominantly in CG poor regions (Bird, A., *Nature*, 321:209, 1986). In contrast, CpG islands remain unmethylated in normal cells, except during X-chromosome inactivation (Migeon, *et al.*, *supra*) and parental specific imprinting (Li, *et al.*, *Nature*, 366:362, 1993) where methylation of 5' regulatory regions can lead to transcriptional repression. *De novo* methylation of the Rb gene has been demonstrated in a small fraction of retinoblastomas (Sakai, *et al.*, *Am. J. Hum. Genet.*, 48:880, 1991), and recently, a more detailed analysis of the VHL gene showed aberrant methylation in a subset of sporadic renal cell carcinomas (Herman, *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, 91:9700, 1994). Expression of a tumor suppressor gene can also be abolished by *de novo* DNA methylation of a normally unmethylated CpG island (Issa, *et al.*, *Nature Genet.*, 7:536, 1994; Herman, *et al.*, *supra*; Merlo, *et al.*, *Nature Med.*, 1:686, 1995; Herman, *et al.*, *Cancer Res.*, 56:722, 1996; Graff, *et al.*, *Cancer Res.*, 55:5195, 1995; Herman, *et al.*, *Cancer Res.*, 55:4525, 1995).

Human cancer cells typically contain somatically altered nucleic acid, characterized by mutation, amplification, or deletion of critical genes. In addition, the nucleic acid from human cancer cells often displays somatic changes in DNA methylation (E.R. Fearon, *et al.*, *Cell*, 61:759, 1990; P.A. Jones, *et al.*, *Cancer Res.*, 46:461, 1986; R. Holliday, *Science*, 238:163, 1987; A. De Bustros, *et al.*, *Proc. Natl. Acad. Sci., USA*, 85:5693, 1988); P.A Jones, *et al.*, *Adv. Cancer Res.*, 54:1, 1990; S.B. Baylin, *et al.*, *Cancer*

*Cells*, 3:383, 1991; M. Makos, *et al.*, *Proc. Natl. Acad. Sci., USA*, 89:1929, 1992; N. Ohtani-Fujita, *et al.*, *Oncogene*, 8:1063, 1993). However, the precise role of abnormal DNA methylation in human tumorigenesis has not been established. Aberrant methylation of normally unmethylated CpG islands has been described as a frequent event in immortalized and transformed cells, and has been associated with transcriptional inactivation of defined tumor suppressor genes in human cancers. In the development of colorectal cancers (CRC), a series of tumor suppressor genes (TSG) such as *APC*, *p53*, *DCC* and *DPC4* are inactivated by mutations and chromosomal deletions (reviewed in Kinzler and Vogelstein 1996). Some of these alterations result from a chromosomal instability phenotype described in a subset of CRC (Lengauer *et al.*, 1997a). Recently, an additional pathway has been shown to be involved in a familial form of CRC, hereditary non-polyposis colorectal cancer. The cancers from these patients show a characteristic mutator phenotype which causes microsatellite instability (MI), and mutations at other gene loci such as *TGF- $\beta$ -RII* (Markowitz *et al.*, 1995) and *BAX* (Rampino *et al.*, 1997). This phenotype usually results from mutations in the mismatch repair (MMR) genes *hMSH2* and *hMLH1* (reviewed by Peltomaki, and de la Chapelle, 1997). A subset of sporadic CRC also show MI, but mutations in MMR genes appear to be less frequent in these tumors (Liu *et al.*, 1995; Moslein *et al.*, 1996).

Another molecular defect described in CRC is CpG island (CGI) methylation. CGIs are short sequences rich in the CpG dinucleotide and can be found in the 5' region of about half of all human genes (Bird, 1986). Methylation of cytosine within 5' CGIs is associated with loss of gene expression and has been seen in physiological conditions such as X chromosome inactivation and genomic imprinting (reviewed in Latham, 1996). Aberrant methylation of CGIs has been detected in genetic diseases

such as the fragile-X syndrome (Hansen *et al.*, 1992), in aging cells (Issa *et al.*, 1994) and in neoplasia. About half of the tumor suppressor genes which have been shown to be mutated in the germline of patients with familial cancer syndromes have also been shown to be aberrantly methylated in some  
5 proportion of sporadic cancers, including *Rb*, *VHL*, *p16*, *hMLH1*, and *BRCA1* (reviewed in Baylin *et al.*, 1998; Jones 1997). TSG methylation in cancer is usually associated with (1) lack of gene transcription and (2) absence of coding region mutation. Thus it has been proposed that CGI methylation serves as an alternative mechanism of gene inactivation in cancer.

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The causes and global patterns of CGI methylation in human cancers remain poorly defined. Aging could play a factor in this process because methylation of several CGIs could be detected in an age-related manner in normal colon mucosa as well as in CRC (Issa *et al.*, 1994). In addition,  
15 aberrant methylation of CGIs has been associated with the MI phenotype in CRC (Ahuja *et al.*, 1997) as well as specific carcinogen exposures (Issa *et al.*, 1996). However, an understanding of aberrant methylation in CRC has been somewhat limited by the small number of CGIs analyzed to date. In fact, previous studies have suggested that large numbers of CGIs are methylated in  
20 immortalized cell lines (Antequera *et al.*, 1990), and it is not well understood whether this global aberrant methylation is caused by the cell culture conditions or whether they are an integral part of the pathogenesis of cancer.

Most of the methods developed to date for detection of methylated  
25 cytosine depend upon cleavage of the phosphodiester bond alongside cytosine residues, using either methylation-sensitive restriction enzymes or reactive chemicals such as hydrazine which differentiate between cytosine and its 5-methyl derivative. Genomic sequencing protocols which identify a 5-MeC

residue in genomic DNA as a site that is not cleaved by any of the Maxam  
Gilbert sequencing reactions have also been used, but still suffer  
disadvantages such as the requirement for large amount of genomic DNA and  
the difficulty in detecting a gap in a sequencing ladder which may contain  
5 bands of varying intensity.

Mapping of methylated regions in DNA has relied primarily on  
Southern hybridization approaches, based on the inability of methylation-  
sensitive restriction enzymes to cleave sequences which contain one or more  
10 methylated CpG sites. This method provides an assessment of the overall  
methylation status of CpG islands, including some quantitative analysis, but is  
relatively insensitive and requires large amounts of high molecular weight  
DNA.

15 Another method utilizes bisulfite treatment of DNA to convert all  
unmethylated cytosines to uracil. The altered DNA is amplified and  
sequenced to show the methylation status of all CpG sites. However, this  
method is technically difficult, labor intensive and without cloning amplified  
products, it is less sensitive than Southern analysis, requiring approximately  
20 10% of the alleles to be methylated for detection.

Identification of the earliest genetic changes in tumorigenesis is a  
major focus in molecular cancer research. Diagnostic approaches based on  
identification of these changes are likely to allow implementation of early  
25 detection strategies and novel therapeutic approaches targeting these early  
changes might lead to more effective cancer treatment.

### **SUMMARY OF THE INVENTION**

The invention provides a method for detecting a methylated CpG-containing nucleic acid. This method can be used to identify sequences which  
5 are differentially methylated during a disease process such as a cell proliferative disorder.

In one embodiment, a method is provided for identifying a methylated CpG-containing nucleic acid. The method includes contacting a nucleic acid  
10 sample suspected of containing a CpG-containing nucleic acid, with a methylation sensitive restriction endonuclease that cleaves only unmethylated CpG sites, under conditions and for a time to allow cleavage of unmethylated nucleic acid; and contacting the sample with an isoschizomer of the  
15 methylation sensitive restriction endonuclease, wherein the isoschizomer of the methylation sensitive restriction endonuclease cleaves both methylated and unmethylated CpG sites. Oligonucleotides are added to the nucleic acid sample under conditions and for a time to allow ligation of the  
oligonucleotides to nucleic acid cleaved by the restriction endonuclease and the digested nucleic acid is amplified for further analysis.

20

In another embodiment, a method is provided for detecting an age-associated disorder associated with methylation of CpG islands in a nucleic acid sequence of interest in a subject having or at risk of having said disorder. The method includes contacting a nucleic acid sample suspected of comprising  
25 a CpG-containing nucleic acid with a methylation sensitive restriction endonuclease that cleaves only unmethylated CpG sites under conditions and for a time to allow cleavage of unmethylated nucleic acid, and contacting the sample with an isoschizomer of the methylation sensitive restriction

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endonuclease, wherein the isoschizomer of the methylation sensitive restriction endonuclease cleaves both methylated and unmethylated CpG sites. Oligonucleotides are added to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotides to nucleic acid cleaved by the restriction endonuclease, and the digested nucleic acid is amplified. The amplified, digested nucleic acid is contacted with a membrane and the membrane is hybridized with a probe of interest.

In yet another embodiment, a method is provided for evaluating the response of a cell to an agent. The method includes contacting a nucleic acid sample suspected of containing a CpG-containing nucleic acid with a methylation sensitive restriction endonuclease that cleaves only unmethylated CpG sites, under conditions and for a time to allow cleavage of unmethylated nucleic acid, and contacting the sample with an isoschizomer of the methylation sensitive restriction endonuclease, wherein the isoschizomer of the methylation sensitive restriction endonuclease cleaves both methylated and unmethylated CpG sites. Oligonucleotides are added to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotides to nucleic acid cleaved by the restriction endonuclease, and the digested nucleic acid is amplified. The amplified, digested nucleic acid is adhered to a membrane and the membrane is hybridized with a probe of interest.

In a further embodiment, a kit for the detection of a methylated CpG-containing nucleic acid is provided. In one embodiment the kit includes a carrier means containing one or more containers including a container containing an oligonucleotide for ligation of the oligonucleotides to nucleic acid, a second container containing a methylation sensitive restriction

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endonuclease and a third container containing an isoschizomer of the methylation sensitive endonuclease. In another embodiment the kit includes a carrier means containing one or more containers containing a membrane, wherein the membrane has a member of the group consisting SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33 (MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, and MINT33 immobilized on the membrane.

In a further embodiment, an isolated nucleic acid including a member selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33 (MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, and MINT33) is provided. An isolated methylated nucleic acid sequence having a sequence as set forth in a member of the group consisting of SEQ ID NOs:1-33 (MINT1-33) is also provided.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1 is a schematic diagram of MCA. A hypothetical fragment of genomic DNA is represented by a solid line, with 7 SmaI sites depicted by tick marks. Methylated SmaI sites are indicated by an m. Fragments B and D are CpG islands. B is methylated in both normal (right) and cancer (left), while D is differentially methylated in cancer. For MCA, unmethylated SmaI sites are eliminated by digestion with SmaI (which is methylation-sensitive and does not cleave when its recognition sequence CCCGGG contains a methylated CpG), which leaves the fragment blunt ended. Methylated SmaI sites are then digested with the non-methylation sensitive SmaI isoschizomer XmaI, which digests methylated CCCGGG sites, leaving a CCGG overhang (sticky ends). Adaptors are ligated to these sticky ends, and PCR is performed to amplify the methylated sequences. The MCA amplicons can be used directly in a dot blot analysis to study the methylation status of any gene for which a probe is available (left). Alternatively, MCA products can be used to clone differentially methylated sequence by RDA (right).

FIG. 2 shows the nucleotide sequence of a differentially Methylated Clone, MINT2 obtained by MCA Followed by RDA. The restriction endonuclease sites for SmaI are underlined. Primer sequences used for bisulfite-PCR are also underlined. The restriction endonuclease site for BstUI used to detect methylation after bisulfite PCR is shown by a gray box.

FIG. 3 show a map of the *versican* gene first exon (filled box) and flanking regions. The position of MINT11 is shown by a solid line (on top). CpG sites are indicated below. Location of the primers used for bisulfite-PCR are shown by arrows.

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FIG. 4 is a pictorial representation of global hypermethylation in CRC. Each column represents a separate gene locus. Each row is a primary colorectal cancer (samples above the bold solid line) or polyp (below the bold solid line). Black squares: methylation > 10%. Gray squares: 1-10% methylation. White squares: < 1% methylation. A: GH+MI+, B: GH+MI-, C: GH-MI+, D: GH-MI-, E: GH+, F: GH-. A-D are cancers. E and F are adenomas. MI denotes the presence of microsatellite instability. ND, not done.

FIG. 5 shows a model integrating CGI methylation in colorectal carcinogenesis.

FIGS. 6A-H are the nucleic acid sequences of MINT1-33 (SEQ ID NO: 1-33).

## 15      **DESCRIPTION OF THE PREFERRED EMBODIMENTS**

The present invention provides a method for identifying a methylated CpG-containing nucleic acid called methylated CpG island amplification (MCA). MCA can be used to study methylation in normal and neoplastic cells, and allows rapid screening of nucleic acid samples for the presence of hypermethylation of specific genes. MCA can also be used to clone genes and nucleic acid sequences differentially methylated in normal and abnormal tissues and cells.

It should be noted that as used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the restriction enzyme" includes

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reference to one or more restriction enzymes and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein  
5 have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

10

All publications mentioned herein are incorporated herein by reference in full for the purpose of describing and disclosing the methodologies which are described in the publications which might be used in connection with the presently described invention. The publications discussed above and  
15 throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

20

Any nucleic acid sample, in purified or nonpurified form, can be utilized as the starting nucleic acid or acids, provided it contains, or is suspected of containing, a nucleic acid sequence containing the target locus (e.g., CpG-containing nucleic acid). In general the CpG-containing nucleic acid will be DNA. However, the process may employ, for example, samples  
25 that contain DNA, or DNA and RNA, including messenger RNA, wherein DNA or RNA may be single stranded or double stranded, or a DNA-RNA hybrid may be included in the sample. A mixture of nucleic acids may also be employed. The specific nucleic acid sequence to be detected may be a fraction

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of a larger molecule or can be present initially as a discrete molecule, so that the specific sequence constitutes the entire nucleic acid. It is not necessary that the sequence to be studied be present initially in a pure form; the nucleic acid may be a minor fraction of a complex mixture, such as contained in whole human DNA. The nucleic acid may be contained in a biological sample. Such samples include but are not limited to a serum, urine, saliva, cerebrospinal fluid, pleural fluid, ascites fluid, sputum, stool, or biopsy sample. The nucleic acid-containing sample used for detection of methylated CpG may be from any source including, but not limited to, brain, colon, urogenital, hematopoietic, thymus, testis, ovarian, uterine, prostate, breast, colon, lung and renal tissue and may be extracted by a variety of techniques such as that described by Maniatis, *et al.* (Molecular Cloning: a Laboratory Manual, Cold Spring Harbor, NY, pp 280, 281, 1982).

The nucleic acid of interest can be any nucleic acid where it is desirable to detect the presence of a CpG island. In one embodiment, the CpG island comprises a CpG island located in a gene. A "CpG island" is a CpG rich region of a nucleic acid sequence. The nucleic acid sequence may be, for example, a p16, a Rb, a VHL, a hMLH1, or a BRCA1 gene. Alternatively the nucleic acid of interest can be, for example, a MINT1-33 nucleic acid sequence. However, any gene or nucleic acid sequence of interest containing a CpG sequence can be detected using the method of the invention.

The presence of methylated CpG in the nucleic acid-containing specimen may be indicative of a disorder. In one embodiment, the disorder is a cell proliferative disorder. A "cell proliferative disorder" is any disorder in which the proliferative capabilities of the affected cells is different from the normal proliferative capabilities of unaffected cells. An example of a cell

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proliferative disorder is neoplasia. Malignant cells (*i.e.*, cancer) develop as a result of a multistep process. Specific, non-limiting examples of disorders associated with increased methylation of CpG-islands are colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, uterine cancer,  
5 astrocytoma, glioblastoma, and neuroblastoma.

In another embodiment, the disorder is an age-associated disorder. The term "age-associated disorder" is used to describe a disorder observed with the biological progression of events occurring over time in a subject. Preferably,  
10 the subject is a human. Non-limiting examples of age-associated disorders include, but are not limited to, atherosclerosis, diabetes melitis, and dementia. An age-associated disorder may also be a cell proliferative disorder. Examples of age-associated disorders which are cell proliferative disorders include colon cancer, lung cancer, breast cancer, prostate cancer, and  
15 melanoma, amongst others. An age-associated disorder is further intended to mean the biological progression of events that occur during a disease process that affects the body, which mimic or substantially mimic all or part of the aging events which occur in a normal subject, but which occur in the diseased state over a shorter period of time.

20

In one embodiment, the age-associated disorder is a "memory disorders or learning disorders" which are characterized by a statistically significant decrease in memory or learning assessed over time by the Randt Memory Test (Randt *et al.*, *Clin. Neuropsychol.*, 2:184, 1980), Wechsler  
25 Memory Scale (*J. Psych.*, 19:87-95, 1945), Forward Digit Span test (Craik, *Age Differences in Human Memory*, in: Handbook of the Psychology of Aging, Birren, J., and Schaie, K., Eds., New York, Van Nostrand, 1977), Mini-Mental State Exam (Folstein *et al.*, *J. of Psych. Res.* 12:189-192, 1975),

or California Verbal Learning Test (CVLT) wherein such non-neurodegenerative pathological factors as aging, anxiety, fatigue, anger, depression, confusion, or vigor are controlled for. (See, U.S. Patent No. 5,063,206 for example).

5

If the sample is impure (*e.g.*, plasma, serum, stool, ejaculate, sputum, saliva, cerebrospinal fluid or blood or a sample embedded in paraffin), it may be treated before amplification with a reagent effective for opening the cells, fluids, tissues, or animal cell membranes of the sample, and for exposing the nucleic acid(s). Methods for purifying or partially purifying nucleic acid from a sample are well known in the art (*e.g.*, Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Press, 1989, herein incorporated by reference).

15

In one embodiment, a method is provided for identifying a methylated CpG-containing nucleic acid, including contacting a nucleic acid sample suspected of comprising a CpG-containing nucleic acid with a methylation sensitive restriction endonuclease that cleaves only unmethylated CpG sites under conditions and for a time to allow cleavage of unmethylated nucleic acid. The sample is further contacted with an isoschizomer of the methylation sensitive restriction endonuclease, that cleaves both methylated and unmethylated CpG-sites, under conditions and for a time to allow cleavage of methylated nucleic acid. Oligonucleotides are added to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotides to nucleic acid cleaved by the restriction endonuclease, and the digested nucleic acid is amplified. Following identification, the methylated CpG-containing nucleic acid can be cloned, using method well

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known to one of skill in the art (see Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Press, 1989).

5 A "methylation sensitive restriction endonuclease" is a restriction endonuclease that includes CG as part of its recognition site and has altered activity when the C is methylated as compared to when the C is not methylated. Preferably, the methylation sensitive restriction endonuclease has inhibited activity when the C is methylated (*e.g.*, *SmaI*). Specific non-limiting examples of a methylation sensitive restriction endonucleases include *SmaI*,  
10 *BssHII*, or *HpaII*. Such enzymes can be used alone or in combination. Other methylation sensitive restriction endonucleases will be known to those of skill in the art and include, but are not limited to *SacII*, *EagI*, and *BstUI*, for example. An "isoschizomer" of a methylation sensitive restriction endonuclease is a restriction endonuclease which recognizes the same  
15 recognition site as a methylation sensitive restriction endonuclease but which cleaves both methylated and unmethylated CGs. One of skill in the art can readily determine appropriate conditions for a restriction endonuclease to cleave a nucleic acid (see Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Press, 1989). Without being bound by theory,  
20 actively transcribed genes generally contain fewer methylated CGs than in other genes.

In the method of the invention, a nucleic acid of interest is cleaved with a methylation sensitive endonuclease. In one embodiment, cleavage with  
25 the methylation sensitive endonuclease creates a sufficient overhang on the nucleic acid of interest. Following cleavage with the isoschizomer, the cleavage product can still have a sufficient overhang. An "overhang" refers to nucleic acid having two strands wherein the strands end in such a manner that

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a few bases of one strand are not base paired to the other strand. A "sufficient overhang" refers to an overhang of sufficient length to allow specific hybridization of an oligonucleotide of interest. In one embodiment, a sufficient overhang is at least two bases in length. In another embodiment, the  
5 sufficient overhang is four or more bases in length. An overhang of a specific sequence on the nucleic acid of interest may be desired in order for an oligonucleotide of interest to hybridize. In this case, the isoschizomer can be used to create the overhang having the desired sequence on the nucleic acid of interest.

10

In another embodiment, the cleavage with a methylation sensitive endonuclease results in a reaction product of the nucleic acid of interest that has a blunt end or an insufficient overhang. In this embodiment, an isoschizomer of the methylation sensitive restriction endonuclease can create a  
15 sufficient overhang on the nucleic acid of interest. "Blunt ends" refers to a flush ending of two strands, the sense strand and the antisense strand, of a nucleic acid.

Once a sufficient overhang is created on the nucleic acid of interest, an  
20 oligonucleotide is ligated to the nucleic acid cleaved of interest which has been cleaved by the methylation specific restriction endonuclease. "Ligation" is the attachment of two nucleic acid sequences by base pairing of substantially complementary sequences or by the formation of a covalent bonds between two nucleic acid sequences. An "oligonucleotide" is a nucleic  
25 acid sequence of 2 to 40 bases in length. Preferably the oligonucleotide is from 15 to 35 bases in length. In one embodiment, the oligonucleotide is ligated to the overhang on the nucleic acid sequence of interest by base pairing.

In one embodiment, two oligonucleotides are utilized to form an adaptor. An "adaptor" is a double-stranded nucleic acid sequence with one end that has a sufficient single-stranded overhang at one or both ends such that the adaptor can be ligated by base-pairing to a sufficient overhang on a nucleic acid of interest that has been cleaved by a methylation sensitive restriction enzyme or an isoschizomer of a methylation sensitive restriction enzyme. In one embodiment, two oligonucleotides can be used to form an adaptor; these oligonucleotides are substantially complementary over their entire sequence except for the region(s) at the 5' and/or 3' ends that will form a single stranded overhang. The single stranded overhang is complementary to an overhang on the nucleic acid cleaved by a methylation sensitive restriction enzyme or an isoschizomer of a methylation sensitive restriction enzyme, such that the overhang on the nucleic acid of interest will base pair with the 3' or 5' single stranded end of the adaptor under appropriate conditions. The conditions will vary depending on the sequence composition (GC vs AT), the length, and the type of nucleic acid (see Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Plainview, NY, 1998).

20

Following the ligation of the oligonucleotide, the nucleic acid of interest is amplified using a primer complementary to the oligonucleotide. Specifically, the term "primer" as used herein refers to a sequence comprising two or more deoxyribonucleotides or ribonucleotides, preferably more than three, and most preferably more than 8, which sequence is capable of initiating synthesis of a primer extension product, which is substantially complementary to a nucleic acid such as an adaptor or a ligated oligonucleotide. Environmental conditions conducive to synthesis include the presence of

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nucleoside triphosphates and an agent for polymerization, such as DNA polymerase, and a suitable temperature and pH. The primer is preferably single stranded for maximum efficiency in amplification, but may be double stranded. If double stranded, the primer is first treated to separate its strands  
5 before being used to prepare extension products. In one embodiment, the primer is an oligodeoxyribo-nucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent for polymerization. The exact length of primer will depend on many factors, including temperature, buffer, and nucleotide composition. The  
10 oligonucleotide primer typically contains 12-20 or more nucleotides, although it may contain fewer nucleotides.

Primers of the invention are designed to be "substantially" complementary to each strand of the oligonucleotide to be amplified and  
15 include the appropriate G or C nucleotides as discussed above. This means that the primers must be sufficiently complementary to hybridize with their respective strands under conditions which allow the agent for polymerization to perform. In other words, the primers should have sufficient complementarity with a 5' and 3' oligonucleotide to hybridize therewith and  
20 permit amplification of CpG containing nucleic acid sequence.

Primers of the invention are employed in the amplification process which is an enzymatic chain reaction that produces exponential quantities of target locus relative to the number of reaction steps involved (*e.g.*, polymerase  
25 chain reaction or PCR). Typically, one primer is complementary to the negative (-) strand of the locus and the other is complementary to the positive (+) strand. Annealing the primers to denatured nucleic acid followed by extension with an enzyme, such as the large fragment of DNA Polymerase I

(Klenow) and nucleotides, results in newly synthesized + and - strands containing the target locus sequence. Because these newly synthesized sequences are also templates, repeated cycles of denaturing, primer annealing, and extension results in exponential production of the region (*i.e.*, the target locus sequence) defined by the primer. The product of the chain reaction is a discrete nucleic acid duplex with termini corresponding to the ends of the specific primers employed.

The oligonucleotide primers of the invention may be prepared using any suitable method, such as conventional phosphotriester and phosphodiester methods or automated embodiments thereof. In one such automated embodiment, diethylphosphoramidites are used as starting materials and may be synthesized as described by Beaucage, *et al.* (*Tetrahedron Letters*, 22:1859-1862, 1981). One method for synthesizing oligonucleotides on a modified solid support is described in U.S. Patent No. 4,458,066.

Where the CpG-containing nucleic acid sequence of interest contains two strands, it is necessary to separate the strands of the nucleic acid before it can be used as a template for the amplification process. Strand separation can be effected either as a separate step or simultaneously with the synthesis of the primer extension products. This strand separation can be accomplished using various suitable denaturing conditions, including physical, chemical, or enzymatic means, the word "denaturing" includes all such means. One physical method of separating nucleic acid strands involves heating the nucleic acid until it is denatured. Typical heat denaturation may involve temperatures ranging from about 80° to 105°C for times ranging from about 1 to 10 minutes. Strand separation may also be induced by an enzyme from the class of enzymes known as helicases or by the enzyme RecA, which has helicase

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activity, and in the presence of riboATP, is known to denature DNA. The reaction conditions suitable for strand separation of nucleic acids with helicases are described by Kuhn Hoffmann-Berling (CSH-Quantitative Biology, 43:63, 1978) and techniques for using RecA are reviewed in  
5 C. Radding (*Ann. Rev. Genetics*, 16:405-437, 1982).

When complementary strands of nucleic acid or acids are separated, regardless of whether the nucleic acid was originally double or single stranded, the separated strands are ready to be used as a template for the  
10 synthesis of additional nucleic acid strands. This synthesis is performed under conditions allowing hybridization of primers to templates to occur. Generally synthesis occurs in a buffered aqueous solution, generally at a pH of about 7-9. Preferably, a molar excess (for genomic nucleic acid, usually about  $10^8$ :1 primer:template) of the two oligonucleotide primers is added to the buffer  
15 containing the separated template strands. It is understood, however, that the amount of complementary strand may not be known if the process of the invention is used for diagnostic applications, so that the amount of primer relative to the amount of complementary strand cannot be determined with certainty. As a practical matter, however, the amount of primer added will  
20 generally be in molar excess over the amount of complementary strand (template) when the sequence to be amplified is contained in a mixture of complicated long-chain nucleic acid strands. A large molar excess is preferred to improve the efficiency of the process.

25 The deoxyribonucleoside triphosphates dATP, dCTP, dGTP, and dTTP are added to the synthesis mixture, either separately or together with the primers, in adequate amounts and the resulting solution is heated to about 90°-100°C from about 1 to 10 minutes, preferably from 1 to 4 minutes. After this

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heating period, the solution is allowed to cool to approximately room temperature, which is preferable for the primer hybridization. To the cooled mixture is added an appropriate agent for effecting the primer extension reaction (called herein "agent for polymerization"), and the reaction is allowed to occur under conditions known in the art. The agent for polymerization may also be added together with the other reagents if it is heat stable. This synthesis (or amplification) reaction may occur at room temperature up to a temperature above which the agent for polymerization no longer functions. Thus, for example, if DNA polymerase is used as the agent, the temperature is generally no greater than about 40°C. Most conveniently the reaction occurs at room temperature.

The agent for polymerization may be any compound or system which will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for example, *E. coli* DNA polymerase I, Klenow fragment of *E. coli* DNA polymerase I, T4 DNA polymerase, other available DNA polymerases, polymerase muteins, reverse transcriptase, and other enzymes, including heat-stable enzymes (*i.e.*, those enzymes which perform primer extension after being subjected to temperatures sufficiently elevated to cause denaturation). Suitable enzymes will facilitate combination of the nucleotides in the proper manner to form the primer extension products which are complementary to each locus nucleic acid strand. Generally, the synthesis will be initiated at the 3' end of each primer and proceed in the 5' direction along the template strand, until synthesis terminates, producing molecules of different lengths. There may be agents for polymerization, however, which initiate synthesis at the 5' end and proceed in the other direction, using the same process as described above.

Preferably, the method of amplifying is by PCR, as described herein and as is commonly used by those of ordinary skill in the art. However, alternative methods of amplification have been described and can also be employed.

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Once amplified, the nucleic acid can be attached to a solid support, such as a membrane, and can be hybridized with any probe of interest, to detect any nucleic acid sequence. Several membranes are known to one of skill in the art for the adhesion of nucleic acid sequences. Specific non-limiting examples of these membranes include nitrocellulose (Nitropure) or other membranes used in for detection of gene expression such as polyvinylchloride, diazotized paper and other commercially available membranes such as Genescreen™, Zetaprobe™ (Biorad), and Nytran™. Methods for attaching nucleic acids to these membranes are well known to one of skill in the art. Alternatively, screening can be done in a liquid phase.

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In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (*e.g.*, GC v. AT content), and nucleic acid type (*e.g.*, RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

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An example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderate stringency

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conditions); and 0.1 x SSC at about 68°C (high stringency conditions).

Washing can be carried out using only one of these conditions, *e.g.*, high stringency conditions, or each of the conditions can be used, *e.g.*, for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed.

- 5 However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically. In general, conditions of high stringency are used for the hybridization of the probe of interest.

- 10 The probe of interest can be detectably labeled, for example, with a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme. Those of ordinary skill in the art will know of other suitable labels for binding to the probe, or will be able to ascertain such, using routine experimentation.

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- In one embodiment, representational difference analysis (RDA, see Lisitsyn *et al.*, *Science* 259:946-951, 1993, herein incorporated by reference) can be performed on CpG-containing nucleic acid following MCA. MCA utilizes kinetic and subtractive enrichment to purify restriction endonuclease
- 20 fragments present in one population of nucleic acid fragments but not in another. Thus, RDA enables the identification of small differences between the sequences of two nucleic acid populations. RDA uses nucleic acid from one population as a "tester" and nucleic acid from a second population as a "driver," in order to clone probes for single copy sequences present in (or
- 25 absent from) one of the two populations. In one embodiment, nucleic acid from a "normal" individual or sample, not having a disorder such as a cell-proliferative disorder is used as a "driver," and nucleic acid from an "affected" individual or sample, having the disorder such as a cell proliferative disorder

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is used as a "tester." In one embodiment, the nucleic acid used as a "tester" is isolated from an individual having a cell proliferative disorder such as colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, uterine cancer, astrocytoma, glioblastoma, and neuroblastoma. The nucleic acid used as a "driver" is thus normal colon, normal lung, normal kidney, normal blood cells, normal breast, normal prostate, normal uterus, normal astrocytes, normal glial and normal neurons, respectively. In an additional embodiment, the nucleic acid used as a "driver" is isolated from an individual having a cell proliferative disorder such as colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, uterine cancer, astrocytoma, glioblastoma, and neuroblastoma. The nucleic acid used as a "tester" is thus normal colon, normal lung, normal kidney, normal blood cells, normal breast, normal prostate, normal uterus, normal astrocytes, normal glial and normal neurons, respectively. One of skill in the art will readily be able to identify the "tester" nucleic acid useful with to identify methylated nucleic acid sequences in given "driver" population.

### **SCREENING AGENTS FOR AN EFFECT ON METHYLATION**

The invention provides a method for identifying an agent which can affect methylation. An agent can affect methylation by either increasing or decreasing methylation. The method includes incubating an agent and a sample containing a CpG-containing polynucleotide under conditions sufficient to allow the components to interact, and measuring the effect of the compound on the methylation of the CpG-containing nucleic acid. In one embodiment, the sample is a cell expressing a polynucleotide of interest. In another embodiment, the sample is substantially purified nucleic acid. "Substantially purified" nucleic acid is nucleic acid which has been separated from the cellular components which naturally accompany it, or from contaminating elements such as proteins, lipids, or chemical resins. Substantially pure nucleic acid can be extracted from any cell type, or can be chemically synthesized. Purity can be measured by any appropriate method, such as measuring the absorbance of light (e.g.,  $A_{260}/A_{280}$  ratio).

The nucleic acid can be identified by the methylated CpG island amplification, as described above. The methylation of the polynucleotide in the sample can then be compared to the methylation of a control sample not incubated with the agent. The effect of the agent on methylation of a polynucleotide can be measured by assessing the methylation of the polynucleotide by the methods of the invention. Alternatively, the effect of the agent on methylation of a polynucleotide can be measured by assessing the expression of the polynucleotide of interest. Means of measuring expression are well known to one of skill in the art (e.g., Northern blotting or RNA dot blotting, amongst others).

The agents which affect methylation can include peptides, peptidomimetics, polypeptides, pharmaceuticals, and chemical compounds and biological agents. Psychotropic, antiviral, and chemotherapeutic compounds can also be tested using the method of the invention.

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“Incubating” includes conditions which allow contact between the test agent and the cell of interest. “Contacting” includes in solution and solid phase. The test agent may also be a combinatorial library for screening a plurality of compounds. Agents identified in the method of the invention can be further cloned, sequenced, and the like, either in solution or after binding to a solid support, by any method usually applied to the isolation of a specific DNA sequence. Molecular techniques for DNA analysis (Landegren *et al.*, *Science* 242:229-237, 1988) and cloning have been reviewed (Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Plainview, NY, 1998).

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The sample can be any sample of interest. The sample may be a cell sample or a membrane sample prepared from a cell sample. Suitable cells include any host cells containing a nucleic acid including a CpG island. The cells can be primary cells or cells of a cell line.

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In one embodiment, the agent is incubated with the sample of interest suspected of including a CpG-containing nucleic acid and methylation is evaluated by MCA. Thus, nucleic acid from the sample suspected of including a CpG-containing nucleic acid is contacted with a methylation sensitive restriction endonuclease which cleaves only unmethylated CpG sites under conditions and for a time to allow cleavage of unmethylated nucleic acid. An isoschizomer of the methylation sensitive restriction endonuclease is

also utilized. An oligonucleotide is added to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotide to nucleic acid cleaved by said restriction endonuclease, and the digested nucleic acid is amplified. The digested nucleic acid is adhered to a membrane, and the  
5 membrane is hybridized with a probe of interest. In one embodiment, representation difference analysis can also be performed.

### **KITS**

10 The materials for use in the assay of the invention are ideally suited for the preparation of a kit. Such a kit may comprise a carrier means containing one or more container means such as vials, tubes, and the like, each of the container means comprising one of the separate elements to be used in the method. One of the container means can comprise a container containing an  
15 oligonucleotide for ligation to nucleic acid cleaved by a methylation sensitive restriction endonuclease. One or more container means can also be included comprising a primer complementary to the oligonucleotide. In addition, one or more container means can also be included which comprise a methylation sensitive restriction endonuclease. One or more container means can also be  
20 included containing an isoschizomer of said methylation sensitive restriction enzyme.

In another embodiment, the kit may comprise a carrier means containing one or more container means comprising a solid support, wherein  
25 the solid support has a nucleic acid sequence selected from the group consisting of MINT1-33 immobilized on the solid support. In one embodiment, the solid support is a membrane. Several membranes are known to one of skill in the art for the adhesion of nucleic acid sequences. Specific

non-limiting examples of these membranes include nitrocellulose (Nitropure) or other membranes used in for detection of gene expression such as polyvinylchloride, diazotized paper and other commercially available membranes such as Genescreen™, Zetaprobe™ (Biorad), and Nytran™. The MINT1-33 sequences immobilized on the solid support can then be hybridized to nucleic acid sequences produced by performing the MCA procedure on the nucleic acids of a sample of interest in order to determine if the nucleic acid sequences contained in the sample are methylated.

#### 10 POLYNUCLEOTIDES AND POLYPEPTIDES

In another embodiment, the invention provides isolated MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotides ( SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33, respectively). These polynucleotides include DNA, cDNA and RNA sequences which encode MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polypeptides. It is understood that naturally occurring, synthetic, and intentionally manipulated polynucleotides are included. For example, MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 nucleic acids may be subjected to site-directed mutagenesis. The nucleic acid sequence for MINT1,

MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 also includes antisense sequences, and sequences encoding dominant negative forms of MINT1, MINT2,  
 5 MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33.

The invention provides methylated and unmethylated forms of MINT1,  
 10 MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotides ( SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID  
 15 NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33, respectively). Methylated nucleic acid sequences are also provided which include MINT3, MINT5, MINT 7, MINT11, MINT12, MINT13, MINT16, MINT18, MINT21, MINT25, MINT26, MINT28, and MINT29  
 20 (SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, and SEQ ID NO:29, respectively). It is understood that naturally occurring, synthetic, and intentionally manipulated polynucleotides are included.

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The polynucleotides of the invention includes "degenerate variants" sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon.

Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of a polypeptide encoded by the nucleotide sequence of SEQ ID NOs: 1-33 is functionally unchanged.

5           Specifically disclosed herein are methylated and unmethylated isolated polynucleotide sequences of MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33. Preferably, the nucleotide sequence is SEQ ID NO:1, SEQ ID  
10 NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33, respectively. Specifically disclosed herein are methylated isolated  
15 polynucleotide sequences of MINT3, MINT5, MINT 7, MINT11, MINT12, MINT13, MINT16, MINT18, MINT21, MINT25, MINT26, MINT28, and MINT29. Preferably, the nucleotide sequence is SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, and SEQ ID NO:29, respectively. The term "polynucleotide"  
20 or "nucleic acid sequence" refers to a polymeric form of nucleotides at least 10 bases in length. By "isolated polynucleotide" is meant a polynucleotide that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in  
25 the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a

separate molecule (*e.g.*, a cDNA) independent of other sequences. The nucleotides of the invention can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single and double forms of DNA.

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The polynucleotide encoding MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 includes SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33, dominant negative forms of MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33, and nucleic acid sequences complementary to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33. A complementary sequence may include an antisense nucleotide. When the sequence is RNA, the deoxynucleotides A, G, C, and T of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33 are replaced by ribonucleotides A, G, C, and U, respectively. Also included in

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the invention are fragments of the above-described nucleic acid sequences that are and are at least 15 bases in length, which is sufficient to permit the fragment to selectively hybridize to DNA that encoded by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33 under physiological conditions or a close family member of MINT1, MINT2, MINT4, MINT6, MINT8; MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33. The term "selectively hybridize" refers to hybridization under moderately or highly stringent conditions which excludes non-related nucleotide sequences. Hybridization conditions have been described above.

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The MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, and MINT33 nucleotide sequence includes the disclosed sequence and conservative variations of the polypeptides encoded by MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotides. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for

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asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

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MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 nucleic acid sequences can be expressed *in vitro* by DNA transfer into a suitable host cell.

10 "Host cells" are cells in which a vector can be propagated and its DNA expressed. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is  
15 used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In one aspect, the MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22,  
20 MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotide sequences may be inserted into an expression vector. The term "expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the sequence of interest genetic sequences. Polynucleotide sequence which encode sequence  
25 of interest can be operatively linked to expression control sequences. "Operatively linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. An expression control sequence operatively linked to a coding

sequence is ligated such that expression of the coding sequence is achieved under conditions compatible with the expression control sequences. As used herein, the term "expression control sequences" refers to nucleic acid sequences that regulate the expression of a nucleic acid sequence to which it is operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (*i.e.*, ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

By "promoter" is meant minimal sequence sufficient to direct transcription. Also included in the invention are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters, are included in the invention (see, *e.g.*, Bitter *et al.*, *Methods in Enzymology* 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage  $\gamma$ , plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. When cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from

mammalian viruses (*e.g.*, the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences of the invention.

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In the present invention, the MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotide sequence may be inserted into an expression vector which contains a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg *et al.*, *Gene* 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.* 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (*e.g.*, T7, metallothionein I, or polyhedron promoters).

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MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotide sequences can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and

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plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention.

5           By "transformation" is meant a genetic change induced in a cell following incorporation of new DNA (*i.e.*, DNA exogenous to the cell). Where the cell is a mammalian cell, the genetic change is generally achieved by introduction of the DNA into the genome of the cell (*i.e.*, stable).

10           By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding sequence of interest. Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as  
15   *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the  $\text{CaCl}_2$  method using procedures well known in the art. Alternatively,  $\text{MgCl}_2$  or  $\text{RbCl}$  can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

20           When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be  
25   cotransformed with DNA sequences encoding the sequence of interest, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to

transiently infect or transform eukaryotic cells and express the protein (see for example, Eukaryotic Viral Vectors, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

5           Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

10           In one embodiment, the invention provides substantially purified polypeptide encoded by MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 and MINT33 polynucleotide sequences. The term "substantially purified" as used herein  
15           refers to a polypeptide which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify a polypeptide encoded by MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30,  
20           MINT31, MINT32 and MINT33 polynucleotide sequence using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24,  
25           MINT27, MINT30, MINT31, MINT32 and MINT33 polypeptide can also be determined by amino-terminal amino acid sequence analysis.

Minor modifications of the MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, and MINT33 primary amino acid sequences may result in proteins which have substantially equivalent activity as compared to the unmodified counterpart polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity still exists.

The polypeptides of the invention also include dominant negative forms of the MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 polypeptide which do not have the biological activity of MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 polynucleotide sequence. A "dominant negative form" of MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, or MINT33 is a polypeptide that is structurally similar to MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 polypeptide but does not have wild-type MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 function. For example, a dominant-negative MINT1, MINT2,

MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 polypeptide may interfere with wild-type MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, 5 MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 function by binding to, or otherwise sequestering, regulating agents, such as upstream or downstream components, that normally interact functionally with the MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, 10 MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32 or MINT33 polypeptide.

### EXAMPLES

The following examples are intended to illustrate but not to limit the 15 invention in any manner, shape, or form, either explicitly or implicitly. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

#### EXAMPLE 1

##### 20 DETECTION OF METHYLATED CPG ISLANDS USING MCA

The principle underlying MCA involves amplification of closely spaced methylated SmaI sites to enrich for methylated CGIs. The MCA technique is outlined in Figure 1A. About 70 to 80% of CpG islands contain at least two closely spaced (<1kb) SmaI sites (CCCGGG). Only those SmaI 25 sites within these short distances can be amplified using MCA, ensuring representation of the most CpG rich sequences. Briefly, DNA is digested with SmaI, which cleaves only unmethylated sites, leaving blunt ends between the C and G. DNA is then digested with the SmaI isoschizomer XmaI, which

does cleave methylated CCCGGG sites, and which leaves a 4 base overhang. Adaptors are ligated to this overhang, and PCR is performed using primers complementary to these adaptors. The amplified DNA is then spotted on a nylon membrane and can be hybridized with any probe of interest.

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As a model experiment, amplification of the *p16* gene CGI was examined because (1) hypermethylation of this CGI in cancer is well characterized, and correlates with silencing of the gene (Herman *et al.*, 1995), and (2) this CGI contains two closely spaced SmaI sites (400bp) which can be amplified by MCA. Initially, the reaction was optimized by testing different primers with a variable GC content, and different PCR conditions. As shown in Figure 1B, using primers with a 70% GC content, the *p16* CGI is amplified strongly in the Caco2 cell line, where it is known to be hypermethylated, while no signal above background was detected from any normal colon mucosa. To examine the quantitative aspect of MCA, DNA from Caco2 and normal colon mucosa were mixed in various proportions, and the methylation level of each mix was determined using MCA. MCA detected *p16* methylation in a semi-quantitative manner between 1% and 100% methylated alleles. Finally, MCA was performed on 109 samples of normal colonic mucosa and adjacent primary colorectal tumor that had previously been typed for *p16* methylation by Southern blot analysis (Ahuja *et al.*, 1997). MCA and Southern blot were concordant in 107/109 (98%) of the cases. In one case, MCA detected a low level of methylation (5-10 %) in a cancer sample that had been judged negative by Southern blot. In the other discordant case (positive by MCA, negative by Southern blot), the discordance may be related to heterogeneous *p16* methylation, as has been described (Costello *et al.*, 1996).

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MCA is a novel PCR-based technique that allows for the rapid enrichment of hypermethylated CG rich sequences, with a high representation of methylated CpG islands. This technique can have several potential applications. MCA is very useful for the determination of the methylation status of a large number of samples at multiple loci simultaneously. By optimizing the PCR conditions, it should be readily adaptable to the study of the methylation status of any gene that has two closely spaced SmaI sites. As shown herein, there is a very high concordance rate between MCA and other methods for the detection of hypermethylation such as Southern blot analysis and bisulfite-based methods. However, MCA (1) requires good quality DNA, excluding the study of paraffin-embedded samples, (2) examines only a limited number of CpG sites within a CGI and (3) is sensitive to incomplete digestion using the methylation-sensitive enzyme SmaI. Nevertheless, many steps in MCA are amenable to automation and, by allowing for the examination of multiple genes relatively quickly, may have important applications in population-based studies of CGI methylation.

## **EXAMPLE 2**

### **IDENTIFICATION OF DIFFERENTIALLY METHYLATED CG IN CRC BY MCA/ RDA**

To identify novel CGIs aberrantly methylated in CRC, RDA (Lisitsyn *et al.*, 1993) was performed on MCA amplicons from the colon cancer cell line Caco2 as a tester, and a mixture of DNA from the normal colon mucosa of 5 different men (to avoid cloning polymorphic SmaI sites or inactive and methylated X chromosome genes from women) as a driver. Two separate experiments were conducted, one using a lower annealing temperature (72/C), and the other using a higher annealing temperature (77/C) and more GC rich primers. After two rounds of RDA, the PCR products were cloned, and

colonies containing inserts were identified by PCR. Based on initial experiments, we expected most of the recovered clones to contain Alu repetitive sequences, which are CG rich and hypermethylated (Kochanek *et al.*, 1993). All clones were therefore probed with an Alu fragment, and only  
5 non-hybridizing clones were analyzed further. Out of 160 non-Alu clones, 46 were independent clones and 33 of these (MINT1-33, Methylated in Tumors, SEQ ID NOs:1-33, respectively) appeared to be differentially methylated in Caco2 cells by comparing hybridization to MCA products from Caco2 and normal colon (Figure 1C). 19 of the clones (MINT1-19) were obtained using  
10 the lower annealing temperature, and 14 (MINT 20-33) using the higher temperature.

To confirm the aberrant methylation of these clones, Southern blot analysis was performed using DNA digested with SmaI or XmaI. All of the  
15 33 clones were hypermethylated in Caco2 compared to normal colon mucosa. Of these 33, one clone (MINT13) detected highly repeated sequences and two clones (MINT18 and MINT28) appeared to correspond to mildly repeated gene families (data not shown). All others appeared to detect single copy DNA fragments. In addition, hypermethylation at CpG sites within the clones  
20 and distinct from the SmaI sites was confirmed by bisulfite-PCR for 6 clones. In each case, Caco2 was found to be hypermethylated at these sites.

By DNA sequencing (example shown in Figure 2), we found that 29 clones had a GC content greater than 50%, and satisfied the minimal criteria  
25 for CGIs (200bp, GC content>50%, CpG/GpC>0.5) (Gardiner-Garden and Frommer, 1987). As might be expected, clones obtained with the higher annealing temperature and more GC rich primers had a relatively higher GC content (Table 1). The size of each clone, percentage of GC nucleotide,

observed/expected CGs, sequence homology and, chromosomal location are summarized in Table 1. MINT5, MINT8, MINT11, MINT14 and MINT16 contained GC rich regions only in one end of the clones, and these may have been recovered from the edge of CGIs.

Table 1: Summary of the 33 Differentially Methylated Clones Isolated by MCA-RDA

Clone	Size (bp)	%GC	O/E	CGI	Blast Homology	Chromosome Map	Methylation Pattern
MINT1	528	56	0.6	Yes	None	5q13-14	Type C
MINT2	562	50	0.8	Yes	None	2p22-21	Type C
MINT3	563	55	1	Yes	Human EST AA557808	1p34-35	Type a
MINT4	481	60	0.8	Yes	None	15q25-26	Type a
MINT5	852	46	0.5	Yes*	Human CpG clone 88c1	14q21-22	Type a
MINT6	401	59	0.6	Yes	None	12q14-15	Type a
MINT7	481	49	0.9	Yes	Human genomic DNA	6p21-22	Type A
MINT8	617	46	0.5	Yes*	None	N.D	Type A
MINT9	605	54	0.3	No	None	1p34-35	Type A
MINT10	608	49	0.6	Yes	None	9q34-Ter	Type A
MINT11	637	49	0.6	Yes*	Versican	5q12-13	Type A
MINT12	552	49	0.6	Yes	CpG clone 33h2	7q31-32	Type C
MINT13	308	60	0.9	Yes	LINE1	N.D	Cell line
MINT14	620	54	0.4	Yes*	None	10p13-15	Type A
MINT15	641	53	0.7	Yes	None	11p12-13	Type A
MINT16	664	62	0.5	Yes*	Alpha-tubulin	2q	Type A
MINT17	491	54	0.7	Yes	None	6	Type C
MINT18	435	58	0.1	No	Acrogranin	N.D	Cell line
MINT19	443	55	0.2	No	None	N.D	Type A
MINT20	510	67	0.8	Yes	mouse OTP	N.D	Type A
MINT21	411	62	0.4	No	None	22q13	Type A
MINT22	438	60	0.9	Yes	None	10p12	Type A
MINT23	346	64	0.8	Yes	Csx	5q34-35	Type A
MINT24	525	63	0.7	Yes	None	3p25-26	Type A
MINT25	339	60	0.7	Yes	Human genomic DNA	22q11	Type C
MINT26	591	58	0.8	Yes	CpG clone 73e1	7q11	Type A
MINT27	242	74	0.7	Yes	None	N.D	Type C
MINT28	463	58	1	Yes	Ribosomal RNA gene	N.D	Type A
MINT29	429	60	0.7	Yes	CpG clone 20b1	7q11	N.D
MINT30	536	65	0.5	Yes	None	20q11	Type A
MINT31	673	65	0.8	Yes	None	17q21	Type C
MINT32	464	66	1	Yes	None	20q13	Type A
MINT33	139	65	0.8	Yes	None	N.D	N.D

O/E : Observed/expected numbers of CpGs. N.D : not determined.

\* Only one portion of the clones has a CpG island.

By DNA homology search using the BLAST program (BLAST 2.0, default parameters, see <http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-newblast?J-form=0>), 4 clones were identical to human gene sequences, four clones were identical to CGIs randomly sequenced from a CGI library (Cross *et al.*, 1994), one was identical to an EST, two clones were identical to high throughput genomic sequences deposited in Genbank, three clones had significant homology to other genes and the other 19 had no significant match in the database; MINT11 was identical to exon 1 and intron 1 of the human *versican* gene (Zimmerman *et al.*, 1989), and corresponded to the 3' edge of a promoter associated CGI; MINT14 was identical to exon 1 of the human *alpha-tubulin* gene (Dobner, P.R., *et al.*, 1987), and was also the 3' edge of the CGI; MINT 24 corresponded to the 3' noncoding region of the human homeobox gene *CSX* (Turbay *et al.*, 1996); MINT21 had a region with 94% homology at the nucleotide level to exon 2 of the mouse *OPT* gene (Simeone *et al.*, 1994) and probably represents the human homologue of this gene; MINT28 was homologous to ribosomal gene sequences; MINT18 was homologous to the acrogranin gene family. To examine the presence of potential promoter sequences in these clones, promoter prediction was performed using several computer programs (see programs available at <http://dot.imgen.bcm.tmc.edu:9331/seq-search/gene-search.html>). Twenty out of the 33 clones were predicted as promoters using the NNPP program, and 6 were predicted as promoters by using the TSSG program.

The chromosomal position of most of the unknown clones was determined using a somatic cell hybrid panel and a radiation hybrid panel (Table 1). Of note, MINT3 and MINT9 mapped to chromosome 1p35-36, MINT13 mapped to 7q31, MINT24 mapped to 3p25-26, MINT25 mapped to

22q11-Ter, and MINT31 mapped to 17q21. All of these chromosomal segments are areas that are frequently deleted in various tumors.

An important application of MCA is in the discovery of novel genes  
5 hypermethylated in cancer. As demonstrated here, MCA coupled with RDA is a rapid and powerful technology for this purpose, and compares favorably with other described techniques (Hayashizaki *et al.*, 1994, Gonzalgo *et al.*, 1997; Huang *et al.*, 1997). In addition to the identification of genes  
10 hypermethylated in cancer, MCA could potentially be used to discover novel imprinted genes using parthenogenetic DNA (Kaneko-Ishino *et al.*, 1995), as well as novel X-chromosome genes.

### EXAMPLE 3

#### 15 SILENCING OF THE *VERSICAN* GENE IN CRC

To determine whether some of these clones truly represented genes silenced by methylation, we examined the *versican* gene in more detail. Versican is a secreted glycoprotein that appears to be regulated by the *Rb* tumor suppressor gene (Rohde *et al.*, 1996). MINT11 corresponds to part of  
20 exon 1 and part of intron 1 of the *versican* gene (Figure 3A). Hypermethylation of the two SmaI sites in exon 1 and intron 1 in colon cancer cell lines was confirmed by both Southern blot analysis and MCA. In order to determine if this methylation was representative of the entire CGI, including the proximal promoter, PCR was performed on bisulfite-treated DNA using primers  
25 designed to amplify the region around the transcription start site of this gene. The PCR product was then digested with restriction enzymes that distinguish methylated from unmethylated DNA. The *versican* promoter was found to be completely methylated in the colon cancer cell lines, DLD1, LOVO, SW48

and SW837, and partially methylated in HCT116 and HT29 (Figure 3B). In primary colon tumors, *versican* was hypermethylated in 17 out of 25 cases (68%). Interestingly, some methylation of the *versican* promoter was also found in normal tissues, albeit at lower levels when compared to tumors. The level of methylation in normal colon mucosa increased with age of the patient (Fig3C), from an average of 6.9% in patients between 20 and 30 years of age, to an average of 28.9% in patients over 80. A linear regression analysis revealed a significant association between age and *versican* promoter methylation ( $R=0.7$ ,  $P<0.000001$ ). Using RT-PCR, we next examined the expression of *versican* in normal colon mucosa and CRC cell lines. *Versican* was found to be expressed in normal colon epithelium, but was markedly down-regulated or absent in methylated colon cancer cell lines. Expression of *versican* in all these cell lines was easily restored after treatment with the demethylating agent, 5-aza-deoxycytidine. These data suggest that *versican* becomes methylated in normal colon in an age-dependent manner, and that this leads to hypermethylation and loss of expression in most colorectal tumors.

Using MCA/RDA 33 differentially methylated clones were identified and characterized in detail. By sequencing, we found that 29 out of the 33 clones satisfy the criteria of CpG islands, demonstrating that MCA can represent CGIs specifically. Of these 29 clones 5 were already known genes (*versican*, *alpha-tubulin*, *CSX*, *OPT* homologue and *ribosomal RNA* gene). Of these, *versican* is most interesting in that this proteoglycan is an Rb inducible gene (Rohde *et al.*, 1996), suggesting that down regulation of this gene product may have an important role in colorectal carcinogenesis, where Rb mutations are rare. The data clearly show that aberrant methylation of the *versican* gene promoter is correlated with silencing of this gene. In addition,

methylation of the alpha-tubulin gene in Caco2 is consistent with the results of studying the gene expression profile of colorectal cancers using SAGE (Zang *et al.*, 1997), which demonstrated that alpha-tubulin is markedly down-regulated in CRC. Methylation of the CSX and OPT genes does not  
5 coincide with their 5' end, and is therefore not expected to silence these genes. It is possible, however, that these CpG islands are associated with alternate transcripts of the genes, or with other nearby genes, which would then be silenced by methylation (Wutz, A., *et al.*, 1997). Finally, methylation of ribosomal genes has previously been seen in aging tissues (Swisshelm, K., *et al.*, 1990) and therefore is not surprising to find in cancers. Because some of  
10 the clones recovered are in the exon 1 region of expressed genes, identification of new tumor suppressor genes might be facilitated by using MCA/RDA clones as probes for screening cDNA library. Indeed, based on their chromosome location, several clones map to chromosomal regions thought to harbor TSGs because they are highly deleted in various tumors (*e.g.*,  
15 chromosome 1p35, 3p25-26, 7q31, 17q21 and 22q11-Ter).

#### **EXAMPLE 4**

##### **TWO TYPES OF METHYLATION IN CRC**

20 By examining the methylation status of several known genes in colorectal tumors, it has been previously demonstrated that some genes tend to be methylated in an age-dependent manner in normal colon (Issa *et al.*, 1994), and are frequently methylated in CRC, while others are methylated in cancers exclusively (Ahuja *et al.*, 1997). To examine this issue on a genome wide  
25 level in some detail, the methylation profile of 31 MINT clones in a panel of colorectal tumors and corresponding normal colon mucosa was examined using MCA (two clones could not be accurately studied because of high background (MINT29) or small size (MINT33)). Because all of these clones

were recovered from a CRC cell line, there was an initial concern that many of these were not representative of methylation in primary (uncultured) tumors. However, of the 31 clones, 29 were also found to be methylated in some primary CRC. The two clones methylated only in the cell line Caco2 were (1) 5 MINT14, a LINE element, and (2) MINT18, a sequence that had a very low CpG frequency and did not qualify as a CGI. Thus, all non-repetitive CGIs recovered were methylated in primary CRC as well as cell lines.

Hypermethylation patterns of these 29 clones fell into two distinct categories. A majority of the clones (22 out of 29) were found to be frequently methylated 10 (>70%) in the tumors tested, and a slight amount of methylation was also detected in normal colon mucosa. For all of these clones, the normal colon mucosa obtained from young patients showed less methylation compared to the normal mucosa from older patients (Figure 4B). Thus, the majority of CGIs hypermethylated in CRC are methylated in normal colon mucosa as 15 well, in an age related manner. This methylation was named Type A for aging-specific methylation.

The remaining 7 clones were methylated exclusively in CRC, and their frequency of methylation was significantly lower than type A methylation 20 (ranging from 10% to 50%). This type of methylation was named type C for cancer-specific.

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Recently, several reports have suggested that aberrant methylation of CGIs may play an important role in cancer development (Baylin *et al.*, 1998; Jones 1997). However, there is little integrated information on aberrant CGI methylation in cancer at multiple loci, probably because of the lack of a  
5 method to detect methylation in a large number of samples for unselected CGIs throughout the genome. Furthermore, it has been shown that cultured cell lines have a high degree of CGI methylation (Antequera *et al.*, 1990) but it was not known to what extent this reflects methylation in primary cancers. To address these issues, the relatively quantitative and high output features of  
10 MCA allowed us to determine the methylation profile of 31 differentially methylated loci in a panel of colorectal carcinomas.

Despite the fact that all sequences were initially recovered from a colon cancer cell line, only 2 out of the 31 clones showed cell line restricted  
15 methylation. From the sequence data, one of these two clones was a repeated sequences (LINE1), and the other was not a CGI. Thus most of the single copy clones recovered proved to be methylated not only in cell lines but also in some primary colon cancers. Analysis of these 29 clones revealed two distinct types of hypermethylation in cancer (Type A for aging and Type C for  
20 cancer), which may have distinct causes, and different roles in cancer development. Type A methylation was seen in the majority of these clones: 22 of 29 (74%) clones were methylated in an age-related manner in normal colon tissue, and hypermethylated at a high frequency in CRC, as we have shown for the ER gene (Issa *et al.*, 1994) and others (Issa *et al.*, 1996; Ahuja *et al.*, submitted). These results suggest that a large number of CGIs in the  
25 human genome are incrementally methylated during the aging process and, for many genes, this methylation correlates with reduced gene expression as shown for ER (Issa *et al.*, 1994) and versican. Although the mechanism of

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Type A methylation is unknown, it is likely to result from physiological processes rather than a genetic alteration because (1) it is very frequent and affects large numbers of cells, (2) it is present in all individuals, not just patients with cancer and (3) this process is gene and tissue specific (Ahuja *et al.*, submitted). Because the methylation status at a given CGI is thought to be related to positive (methylator) factors (Mummaneni *et al.*, 1993; Mummaneni *et al.*, 1995; Magewu and Jones, 1994, Vertino *et al.*, 1996) and negative (protector) factors (Macleod *et al.*, 1994; Brandeis *et al.*, 1994; Turker and Bestor, 1997; Chen *et al.*, 1997), it is possible that for some genes, this balance favors slightly de-novo methylation, and that this is reflected by progressive hypermethylation after repeated cell divisions.

#### **EXAMPLE 5**

##### **GLOBAL HYPERMETHYLATION IN CRC**

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To understand the patterns of cancer-specific methylation in CRC, the methylation status of all 7 type C clones was analyzed, as well as *p16* in primary cancers and polyps (Figure 4). Two of these clones (MINT1 and MINT2) were studied by both MCA and bisulfite-PCR, and the concordance between the two techniques was found to be 98%. *P16* was studied by both MCA and Southern blot, with a concordance rate of 98%. When we considered the six clones that were methylated in more than 10% of the cases, as well as *p16*, a remarkable pattern emerged (summarized in Figure 5 and Table 2).

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Table 2: Percentage of Tumors Methylated at Multiple MINT Loci, *p16* and *hMLH1*.

	Type C Methylation										Type A Methylation									
	MINT	MINT	MINT	MINT	MINT	MINT	MINT	<i>p16</i>	<i>hMLH</i>		MINT	MINT	MINT	MINT	MINT	MINT	MINT	MINT	MINT	MINT
	1	2	12	17	25	31			1		6	8	22	24	30	32				
MI+ Cancer																				
EI+ (n=12)	100%	100%	100%	92%	100%	92%	83%	100%			100%	100%	100%	100%	100%	100%				
EI- (n=4)	25%	0%	0%	0%	0%	0%	0%	25%			100%	100%	100%	75%	75%	50%				
MI- Cancer																				
EI+ (n=17)	65%	82%	59%	41%	100%	82%	59%	0%			94%	100%	100%	100%	60%	93%				
EI- (n=17)	0%	12%	0%	0%	12%	0%	0%	0%			82%	86%	100%	82%	40%	65%				
Adenoma																				
EI+ (n=7)	86%	86%	71%	29%	86%	71%	57%	0%			86%	83%	100%	100%	43%	86%				
EI- (n=8)	0%	0%	0%	0%	0%	13%	0%	0%			50%	100%	86%	50%	13%	25%				

The 50 CRC fell into two distinct groups: (1) A group with a high level of Type C methylation, whereby all the tumors had methylation of 4 or more loci simultaneously and (2) a group where methylation of any type C clone is extremely rare. Thus, the first group of tumors appears to display profound global hypermethylation (GH+), which is lacking in the second group (GH-). Interestingly, there was a great concordance between methylation of the *p16* gene, which was not selected for by our cloning process, and the presence of GH. In sharp contrast, Type A methylation was not significantly different between GH+ and GH- tumors (Table 2).

GH was also detected in a subset of colorectal adenomas (Figure 5), suggesting that it is an early event in carcinogenesis. Interestingly, while 5 of 5 small adenomas (<7 mm) were GH-, 6 of 9 large adenomas (>10 mm) were GH+, suggesting that this defect may be acquired in the transition between small and large adenomas. In 6 cases, both an adenoma and a cancer from the same patients were examined. In one of these, GH was detected both in the adenoma and the cancer; in 3 cases, GH was detected in the cancer but not in the adenoma and in 2 cases, GH was detected in neither the adenoma nor the cancer.

By contrast to type A methylation, type C methylation is relatively infrequent in primary CRC, and is never observed in normal colon mucosa. Furthermore, detailed analysis of type C methylation in CRC revealed a striking pattern, suggesting the presence of global hypermethylation in a subset of these tumors: GH positive cases are characterized by frequent and concordant methylation of all type C clones examined, such that each tumor has at least four methylation events. By contrast, type C methylation is

virtually non-existent in tumors without GH. This concordance cannot be due to simple experimental variation or artifacts because (1) methylation was verified using separate methods (MCA, bisulfite-PCR and Southern blots), (2) the concordance was not limited to MCA/RDA derived clones since it also

5 affected the p16 (Herman *et al.*, 1995) and hMLH1 (Kane *et al.*, 1997) genes, and (3) there was no significant difference in type A methylation between GH+ and GH- tumors. Global hypermethylation appears to be an early event in the development of CRC, being detectable in large pre-neoplastic adenomas. Because many genes are potential candidates for inactivation

10 through promoter methylation (Baylin *et al.*, 1998; Jones, 1996), global hypermethylation may have profound pathophysiologic consequences in neoplasia through the simultaneous inactivation of tumor-suppressor genes (such as p16), metastasis-suppressor genes (such as E-cadherin), angiogenesis inhibitors (such as Thrombospondin-1) and others. In fact, our data suggest

15 that global hypermethylation could also result in mismatch repair deficiency through methylation and inactivation of the hMLH1 promoter, and may explain up to 75% of cases of sporadic CRC with microsatellite instability. The causes of type A and type C methylation are probably different because the latter is detected only in a limited number of cases, and the genes affected

20 are different. Because of the remarkable concordance in type C methylation among GH+ cases, it appears likely that these tumors all share a specific defect in the maintenance of the methylation-free state in CGIs. This defect could be either aberrant de-novo methylation (through a mutation in DNA-methyltransferase for example), or loss of protection against de-novo

25 methylation, through the loss of a trans-activating factor (Macleod *et al.*, 1994; Chen *et al.*, 1997). Because DNA-methyltransferase activity is similar in the two groups, the latter hypothesis is more likely. Thus, at least in colorectal cancer, it appears likely that type C methylation (an epigenetic error) is

actually caused by a genetic event that results in an increased chance of methylating a subset of CGIs. Ironically, this epigenetic defect may then result in additional genetic lesions through the induction of mismatch-repair deficiency.

5

**EXAMPLE 6**  
**MICROSATELLITE INSTABILITY IS LINKED TO**  
**GLOBAL HYPERMETHYLATION IN CRC**

10 In a previous study (Ahuja *et al.*, 1997), a link was reported between microsatellite instability and a hypermethylator phenotype in sporadic CRC. Relatively few mutations in mismatch repair genes have been reported in sporadic MI+ cancers, but *hMLH1* methylation has recently been observed in some cases (Kane *et al.*, 1997). To determine the relation between global

15 hypermethylation and microsatellite instability in CRC, we measured *hMLH1* methylation using bisulfite/PCR in our panel of CRC which had also been previously typed for the presence of microsatellite instability (Figure 5). *hMLH1* was studied by bisulfite-PCR only because it does not have 2 SmaI sites in its CGI. Overall, 16 out of 50 (32%) cancers had evidence of

20 microsatellite instability. Among the 29 GH+ cases, 12 had evidence of *hMLH1* methylation, suggesting that *hMLH1* is one of the targets of global hypermethylation in CRC. All of these 12 tumors had microsatellite instability. By contrast, *hMLH1* methylation was detected in only one of the 21 GH- cases. These data establish a strong link between the GH phenotype,

25 *hMLH1* methylation and microsatellite instability in CRC. Two lines of evidence suggest that microsatellite instability may follow, and be caused by, global hypermethylation and *hMLH1* methylation. First, GH is detectable in about half of colonic adenomas, but none of these tumors have *hMLH1*

methylation, and microsatellite instability is extremely rare in this pre-neoplastic lesion (Samowitz and Slattery, 1997). Second, GH is not simply caused by mismatch repair defects because microsatellite instability is absent in more than half of the GH+ cases, and GH was absent in 4 of the 16 cancers with microsatellite instability. Overall, our data suggest that, in sporadic CRC, the majority (12 out of 16, or 75%) of cases with microsatellite instability may be caused by GH followed by *hMLH1* methylation, loss of *hMLH1* expression and resultant mismatch repair deficiency (Herman *et al.*, submitted).

10

Based on these data, the following model has been developed integrating CGI methylation into CRC development (Figure 6). In this model, CGI methylation plays two distinct roles, and appears to arise through distinct mechanisms. Initially, type A methylation arises as a function of age in normal colorectal epithelial cells. By affecting genes that regulate the growth and/or differentiation of these cells, such methylation results in a hyperproliferative state, which is thought to precede tumor formation in the colon (reviewed by Lipkin, 1988). Such hyperproliferation is known to arise with age in colorectal epithelium (Holt *et al.*, 1988, Roncucci *et al.*, 1988), and to be marked in patients with CRC. The cause of type A methylation is unknown, but without being bound by theory it is possible that it is related to endogenous factors inherent to the structure of DNA, and that it may be modulated by factors such as level of ongoing expression and exposure to carcinogenic insults. Furthermore, modulation of type A methylation may provide one possible explanation for the reduction in CRC tumorigenesis by reducing levels of DNA-methyltransferase (Laird *et al.*, 1995).

15

20

25

A second major role for CGI methylation appears later, perhaps at the transition between small and large adenomas in the colon. This methylation (type C) affects only a subset of tumors, which then evolve along a pathway of global hypermethylation. This GH leads to cancer development through the simultaneous inactivation of multiple tumor-suppressor genes such as p16, and induction of mismatch repair deficiency through inactivation of hMLH1. The cause of this global hypermethylation is unknown, but may well be related to inactivation of a gene that protects CGIs from de-novo methylation. Finally, we propose that tumors without GH evolve along more classic genetic instability pathways, including chromosomal instability (Lengauer *et al.*, 1997A). Interestingly in this regard, Lengauer et al found an inverse correlation between chromosomal instability and MMR deficiency in CRC cell lines (Lengauer *et al.*, 1997B).

While based on CRC, this model is applicable to most human malignancies. In evidence has also been found for type A and type C methylation in brain tumors (Li *et al.*, 1998). Preliminary evidence also suggests the presence of global hypermethylation in multiple types of cancers, including stomach cancers, brain tumors and hematopoietic malignancies.

In conclusion, a novel method, MCA, has been developed to selectively amplify methylated CGIs. Using MCA/RDA 33 differentially methylated clones in CRC were isolated. The methylation profile of these clones revealed that nearly all methylation in CRC can be accounted for by (1) age-related methylation and (2) a hypermethylator phenotype presumably caused by global hypermethylation. Deciphering the mechanisms underlying these phenomena should facilitate the early detection, prevention and therapy of cancers, including colorectal cancers.

**EXAMPLE 7**  
**IDENTIFICATION OF CACNA1G AS A TARGET FOR**  
**HYPERMETHYLATION ON HUMAN CHROMOSOME 17q21**

5

To identify genes differentially methylated in colorectal cancer, methylated CpG island amplification was used followed by representational difference analysis (Razin and Cedar, *Cell* 17: 473-476, 1994, herein incorporated by reference). One of the clones recovered (MINT31, see above) mapped to human chromosome 17q21 using a radiation hybrid panel, and a Blast search revealed this fragment to be completely identical to part of a BAC clone (Genbank: AC004590) sequenced by high throughput genomic sequence. The region surrounding MINT31 fulfills the criteria of a CpG island: GC content 0.67, CpG/GpC ratio 0.78 and a total of 305 CpG sites in a 4 kb region. Using this CpG island and 10 kb of flanking sequences in a Blast analysis, several regions highly homologous to the rat T-type calcium channel gene, *CACNA1G*, were identified (Perez-Reyes et al., *Nature* 391: 896-900. 1998, herein incorporated by reference). Several ESTs were also identified in this region. Using Genscan, 2 putative coding sequences (G1, and G2) were identified. Blastp analysis revealed that G1 has a high homology to the EH-domain-binding protein, epsin, while G2 is homologous to a C-elegans hypothetical protein (accession No. 2496828).

The MINT31 CpG island corresponds to the 3' regions of G1 and G2, based on the direction of the open reading frame and the presence of a poly A tail, and is unlikely to influence their transcription. The EST closest to MINT31 (H13333) was sequenced entirely and was found not to contain a continuous open reading frame, but a poly-adenylation signal was identified

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on one end, along with a poly A tail. These data suggest that H13333 corresponds to the last 2 exons of an unidentified gene. MINT31 is in the intron of this gene and is unlikely to influence its transcription. However, based on both promoter prediction (TSSG) analysis of this region and  
5 homology to the rat *CACNA1G* sequence, the MINT31 CpG island is also in the 5' region of human *CACNA1G* gene and may play a role in its transcriptional activity.

The human *CACNA1G* sequence deposited in Genbank lacks the 5'  
10 region of the gene, when compared to the rat homologue. To determine the 5' region of human *CACNA1G*, we amplified cDNA by RT-PCR using primers based on the BAC sequence (Genbank: AC004590, herein incorporated by reference). The PCR products were cloned and sequenced, and the genomic organization of the gene was determined by comparing the newly identified  
15 sequences as well as the known sequences to the BAC that covers this region. *CACNA1G* is composed of 34 exons which span a 70 kb area. Based on sequences deposited in Genbank, the gene has two possible 3' ends caused by alternate splicing. *CACNA1G* is highly homologous to rat *CACNA1G* with 93% identity at the protein level, and 89% identity at the nucleotide level. The  
20 5' flanking region of *CACNA1G* lacks TATA and CAAT boxes, which is similar to many housekeeping genes. A putative TFIID binding site was identified 547-556 bp upstream from the translation start site, and several other potential transcription factor binding sites such as AP1 (1 site), AP2 (2 sites) and SP1 (10 sites), were identified upstream of *CACNA1G* exon 1 using  
25 the promoter prediction program, TESS (data not shown).

The *CACNA1G* CpG island is 4 kb, and is larger than many typical CpG islands. MINT31 corresponds to the 5' edge of the island while

*CACNA1G* is in the 3' region. It is not known whether large CpG islands such as this are coordinately regulated with regards to protection from methylation, and aberrant methylation in cancer. To address this issue, the methylation status of the 5' region of *CACNA1G* was studied using bisulfite-PCR of DNA from normal tissues as well as 35 human cancer cell lines from colon, lung, prostate, breast and hematopoietic tumors. The CpG island was divided into 8 regions and their methylation status was examined separately. The genomic DNA was treated with sodium bisulfite and PCR amplified using primers containing no or a minimum number of CpG sites. Methylated alleles were detected by digesting the PCR products using restriction enzymes which specifically cleave sites created or retained due to the presence of methylated CpGs. None of the regions was methylated in normal colon, consistent with a uniform protection against de-novo methylation.

Regions 1 and 2 were frequently methylated in cancer cell lines, and behaved in a concordant manner. These 2 regions were methylated in most cancer cell types except gliomas, and most cell lines where methylation was found methylated both regions simultaneously. Region 3, which is less CG rich than any of the other regions, had either no methylation or very low levels of methylation in most cell lines. Regions 5, 6, and 7 behaved quite differently compared to 1-3. Methylation of these regions was less frequent than regions 1-2, as 22/35 cell lines had no detectable methylation there, despite often showing methylation of region 1-2. However, when methylation was present (in 13/35 cell lines), it affected all 3 regions simultaneously, although to varying extents. Finally, regions 4 and 8 behaved differentially again, being partially methylated primarily in colon and breast cell lines. Therefore, with regards to hypermethylation in cancer, the CpG rich region upstream of *CACNA1G* appears to be composed of 2 CpG islands which

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behave independently. MINT31 corresponds to the upstream CpG island (island 1, regions 1 and 2), while the 5' region of *CACNA1G* is contained in the downstream CpG island (island 2, regions 5-7). Regions 3, 4 and 8 correspond to the edge of these CpG islands, and behave a little differently than the hearts of the CpG islands, as previously described for the *E-Cad* gene (Graff, et al., *J. Biol. Chem.* 272: 22322-22329, 1997).

Overall, the methylation patterns fell into 5 distinct categories: (1) No methylation in any region (normal tissue). (2) Slight methylation of island 1 (6 cell lines, see for example TSU-PRL in Fig. 2). (3) Heavy methylation of island 1 but no methylation of island 2 (16 cell lines, see for example Caco2 in Fig. 2). (4) Heavy methylation of island 1 and moderate to heavy methylation of island 2 (6 cell lines, see for example RKO and Raji in Fig. 2). (5) High methylation of island 1 and low to moderate methylation of island 2 (7 cell lines, see for example MB-231 in Fig. 2).

In a previous study of rat *CACNA1G*, this gene was shown to be expressed most abundantly in the brain (Perez-Reyes et al., *Nature* 391: 896-900, 1998). To determine the expression of *CACNA1G* in normal and neoplastic human cells, RT-PCR was performed using cDNA from various normal tissues and from a panel of 27 tumor cell lines. *CACNA1G* was expressed ubiquitously in a variety of tissues and cell lines. In normal tissues expression was relatively low but easily detectable, while most cell lines had relatively high expression of *CACNA1G*. However, some cell lines had negligible or totally absent levels of *CACNA1G* expression. The results of *CACNA1G* expression was correlated with the detailed methylation analysis previously described. In this analysis, a remarkable pattern emerged. Methylation of region 1-4 and 8 had no effect on *CACNA1G* expression.

However, there was a strong correlation between methylation of regions 5-7 and expression of the gene. In fact, all cell lines that lack methylation of this region strongly express the gene. All 6 cell lines with pattern 4 methylation studied had no detectable expression. Finally, the 7 cell lines with pattern 5 methylation (examples DLD-1 and MB-453) had variable levels of expression ranging from very low to near normal. The fact that patterns 3 and 5 differ significantly with regards to expression, but are almost identical with regards to methylation of all regions except 7 suggests that this area is important in the inactivation of *CACNA1G*.

To confirm whether methylation of the 5' CpG island of *CACNA1G* is really associated with gene inactivation, 3 non-expressing cell lines showing pattern 4 methylation (RKO, SW48 and Raji) and 2 weakly expressing cell lines showing pattern 5 methylation (MB-231 and MB-435) were treated with 1 M of the methyl-transferase inhibitor 5-deoxy-azacitidine. After treatment, all these cell lines re-expressed *CACNA1G* mRNA. Consistent with re-expression, demethylation of region 7 was observed after 5-deoxy-azacitidine treatment (Fig. 3C).

De novo cytosine methylation is thought to sometimes occur in vitro during cell propagation (Antequera et al., *Cell* 62: 503-514, 1990). To determine whether the methylation of *CACNA1G* occurs in vivo, primary human tumors were examined for methylation of the 5' region of *CACNA1G*. Aberrant methylation was detected in 17 out of 49 (35%) colorectal cancers, 4 out of 28 colorectal adenomas (25%), 4 out of 16 (25%) gastric cancers and 3 out of 17 (18%) acute myelogenous leukemia cases. In colorectal cancers, there was a significant correlation between methylation of *CACNA1G* and methylation of *p16* ( $p < 0.005$ ) and *hMLH1* ( $p < 0.001$ ), as well as a strong

correlation with the presence of microsatellite instability, and the recently identified CpG island methylator phenotype (CIMP), supporting that *CACNA1G* is also a target for CIMP in colorectal cancer.

5           To determine whether aberrant methylation of the 5' region of *CACNA1G* affects the expression status of this gene in primary tumors, we performed RT-PCR using cDNA from a series of colorectal adenomas. Six out of 8 cases which showed no methylation of region 7 expressed *CACNA1G*. In sharp contrast, all 5 cases that showed methylation of region 7 had no  
10 detectable expression of this gene.

          Thus, a human T-type calcium channel gene (*CACNA1G*) has been identified and cloned using the MINT31 sequence as a probe. The human T-type calcium channel gene has been determined to be a target of aberrant  
15 methylation and silencing in human tumors. The data show that MINT31 (a representative sequence of MINT1-33) can be used as a probe to identify genes that play a role in disorders such as cell proliferative disorders.

          Detailed analysis of the CpG island upstream of *CACNA1G* revealed  
20 that methylation 300 to 800 bp upstream of the gene closely correlated with transcriptional inactivation. The *CACNA1G* promoter is contained in a large CG rich area that is not coordinately methylated in cancer. The CpG island around MINT31 is much more frequently methylated in cancers compared to that just upstream of *CACNA1G*. This may simply be caused by differential  
25 susceptibility to de-novo methylation between these two regions, with methylation of MINT31 serving as a trigger, and eventually spreading to *CACNA1G*, as described in other genes (Graff, et al., *J. Biol. Chem.* 272: 22322-22329, 1997). However, it is likely that these 2 regions are controlled

by different mechanisms because (1) cell lines kept in culture for countless generations do not in fact spread methylation from MINT31 to *CACNA1G* (e.g., Caco2), (2) region 3 that separates the 2 islands is infrequently and sparsely methylated in cancer and (3) 2 cases of primary colorectal cancer were found which are methylated at the *CACNA1G* promoter but not at MINT31). Therefore, methylation of MINT31 appears to be independent of methylation of *CACNA1G* suggesting that they are 2 distinct CpG islands regulated by different mechanisms. These data leave open the possibility that MINT31 is the promoter for an unidentified gene, which may perhaps be transcribed opposite to *CACNA1G*.

Many CpG islands of silenced genes appear to be methylated uniformly and heavily throughout the island (e.g., Graff, et al., *J. Biol. Chem.* 272: 22322-22329, 1997). In contrast the methylation patterns of the 5' region of *CACNA1G* (region 5-7) was heterogeneous in the cell lines which did not express this gene. Nevertheless, methylation does appear to play a role in *CACNA1G* repression since demethylation readily reactivates the gene.

The causes of *CACNA1G* methylation remain to be determined. Methylation was not detected in normal colon mucosa, placenta, normal breast epithelium and normal bone marrow, including samples from aged patients, suggesting that methylation of this region is cancer specific. However, there was a significant correlation between methylation of *CACNA1G* and other tumor suppressor genes such as *p16* and *hMLH1*. Thus, *CACNA1G* probably is a target for the recently described CIMP phenotype, which results in a form of epigenetic instability with simultaneous inactivation of multiple genes. It should be noted that a gene identified by the method of the invention

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(MINT31) has been successfully utilized to identify another gene of interest (*CACNA1G*) whose methylation pattern correlates with the presence of specific cell proliferative disorders.

5           T-type calcium channels are involved not only in electrophysiological rhythm generation but also in the control of cytosolic calcium during cell proliferation and cell death (reviewed in Berridge, et al., *Nature* 395: 645-648, 1998). The results demonstrate that the expression of *CACNA1G* is not limited to brain and heart, suggesting that it may play a role in these other  
10       tissues. It has previously been shown that  $\text{Ca}^{2+}$  influx via T-type channels is an important factor during the initial stages of cell death such as apoptosis (Berridge, et al., *Nature* 395: 645-648, 1998), ischemia (Fern, *J. Neurosci.* 18: 7232-7243, 1998) and complement-induced cytotoxicity (Newsholme, et al., *Biochem. J.* 295: 773-779, 1993.). These studies determining the methylation  
15       status of the *CACNA1G* suggest that the impairment of voltage gated calcium channels may play an important role in cancer development and progression through altering calcium signaling.

**EXAMPLE 8****EXPERIMENTAL PROCEDURES***Methylated CpG Island Amplification.*

The procedure is outlined in Figure 1. Five µg of DNA were digested  
5 with 100 units of SmaI for 6 hours (all restriction enzymes were from NEB).  
The DNA was then digested with 20 units of XmaI for 16 hours. DNA  
fragments were then precipitated with ethanol. RXMA and RMCA PCR  
adaptors were prepared by incubation of the oligonucleotides RXMA24  
(5'-AGCACTCTCCAGCCTCTCACCGAC-3') (SEQ ID NO: 34) and  
10 RXMA12 (5'-CCGGGTCGGTGA-3') (SEQ ID NO:35), or RMCA24  
(5'-CCACCGCCATCCGAGCCTTTCTGC-3') (SEQ ID NO:36) and  
RMCA12 (5'-CCGGGCAGAAAG-3') (SEQ ID NO:37) at 65°C for two min.  
followed by cooling to room temperature. 0.5 µg of DNA was ligated to  
0.5 nmol of RXMA or RMCA adaptor using T4 DNA ligase (NEB). PCR was  
15 performed using 3 µl of each of the ligation mix as a template in a 100 µl  
volume containing 100 pmol of RXA24 or RMC24 primer, 5 units of Taq  
DNA polymerase, (GIBCO-BRL.), 4 mM MgCl<sub>2</sub>, 16 mM of NH<sub>4</sub> (SO<sub>4</sub>)<sub>2</sub>,  
10mg/ml of BSA, and 5% v/v DMSO. The reaction mixture was incubated at  
72°C at 5 min and at 95°C for 3 min. Samples were then subjected to 25  
20 cycles of amplification consisting of 1 min at 95°C, and 3 min either at 72°C  
or 77°C in a thermal cycler (Hybaid, Inc.). The final extension time was 10  
min.

*Detection of Aberrant Methylation Using MCA.*

25 MCA products from normal colon mucosa and corresponding cancer  
tissues were prepared as described above. One µg of MCA products was  
resuspended in 4 µl of TE (10 mM Tris pH 8.0, 1 mM EDTA pH 8.0), mixed  
with 2 µl of 20 X SSC, and 1 µl aliquot of this mix was blotted onto nylon

membranes (Nunc) using a 96 well replication system (Nunc). The membranes were baked at 80°C, UV crosslinked for 2 min. and hybridized using <sup>32</sup>P labeled probes. Each sample was blotted in duplicate. Each filter included mixtures of a positive control (Caco2) and a negative control (normal colon mucosa from an 18 year old individual). The filters were exposed to a phosphor screen for 24 to 72 hours and developed using a phosphorimager (Molecular Dynamics). The intensity of each signal was calculated using the Image Quant software, and methylation levels were determined relative to the control samples.

10

#### *RDA.*

RDA was performed essentially as previously reported (Lisitsyn *et al.*, 1993) with the following modifications. For the first and second rounds of competitive hybridization, 500 ng and 100 ng of ligation mix was used, respectively. To eliminate the digested adaptor, a cDNA spun column (Amersham) was used instead of excising from the agarose gel. Primers used for the first and second rounds of RDA are as follows :

JXMA24	5'-ACCGACGTCGACTATCCATGAACC-3'	SEQ ID NO:38
JXMA12	5'-CCGGGGTTCATG-3'	SEQ ID NO:39
JMCA24	5'-GTGAGGGTCGGATCTGGCTGGCTC-3'	SEQ ID NO:40
JMCA12	5'-CCGGGAGCCAGC-3'	SEQ ID NO:41
NXMA24	5'-AGGCAACTGTGCTATCCGAGTGAC-3'	SEQ ID NO:42
NXMA12	5'-CCGGGTCACTCG-3'	SEQ ID NO:43
NMCA24	5'-GTTAGCGGACACAGGGCGGGTCAC-3'	SEQ ID NO:44
NMCA12	5'-CCGGGTGACCCG-3'	SEQ ID NO:45

After the second round of competitive hybridization, PCR products were digested with XmaI. The J adaptor was eliminated by column filtration. The PCR products were then subcloned into Bluescript SK(-) (Stratagene). To screen for inserts, a total of 396 clones were cultured overnight in LB medium with ampicillin and 3 µl of the culture was directly used as template for a PCR reaction. Each clone was amplified with

T3 (5'-AATTAACCCTCACTAAAGGG-3') (SEQ ID NO:46)

and

T7 (5'-GTAATACGACTCACTATAGGGC-3') (SEQ ID NO:47)

primers,

blotted onto nylon membranes, and screened for cross hybridization with <sup>32</sup>P labeled inserts. The clones differentially hybridizing to tester and driver MCA products were further characterized by Southern blot analysis and DNA sequencing.

#### *Southern blot analysis.*

Five µg of DNA was digested with 20-100 units of restriction enzymes as specified by the manufacturer (NEB). DNA fragments were separated by agarose gel electrophoresis and transferred to a nylon membrane (Zeta-probe, Bio-Rad). Filters were hybridized with <sup>32</sup>P-labeled probes and washed at 65°C with 2X SSC, 0.1 % SDS for 10 min. twice, and 0.1X SSC, 0.1 % SDS for 20 min. Filters were then exposed to a phosphor screen for 24-72 hours and analyzed by using a phosphorimager (Molecular Dynamics).

#### *DNA sequencing and analysis.*

Plasmid DNA was prepared using the Wizard Plus Minipreps (Promega) according to the suppliers recommendation. Sequence analysis was carried out at the Johns Hopkins Core Sequencing Facility using automated

DNA sequencers (Applied Biosystems). Sequence homologies were identified using the BLAST program of the National Center for Biotechnology Information (NCBI) available at <http://www.ncbi.nlm.nih.gov/BLAST> using the default parameters of the web site. Putative promoter sequences were

5 predicted using the computer programs NNPP and TSSG available through the Baylor college of Medicine launcher at <http://dot.imgen.bcm.tmc.edu:9331>.

*Bisulfite-restriction methylation analysis.*

DNA from colon tumors, cell lines and normal colon mucosa was

10 treated with bisulfite as reported previously (Herman *et al.*, 1996). Primers used for PCR were as follows:

	hMLH1,	5'-TAGTAGTYGTTTTAGGGAGGGA-3' (SEQ ID NO:44),
		5'-TCTAAATACTCAACRAAAATACCTT-3' (SEQ ID NO:45);
15	MINT1,	5'-GGGTTGGAGAGTAGGGGAGTT-3' (SEQ ID NO:46),
		5'-CCATCTAAAATTACCTCRATAACTTA-3' (SEQ ID NO:47);
20	MINT2,	5'-YGTTATGATTTTGTTTAGTTAAT-3' (SEQ ID NO:48), 5'-TACACCAACTACCCAACTACCTC-3' (SEQ ID NO:409);
	Versican,	5'-TTATTAYGTTTTTTATGTGATT-3' (V1) (SEQ ID NO:50), 5'-ACCTTCTACCAATTACTTCTTT-3' (V2) (SEQ ID NO:51).
25		

Ten to 20 µl of the amplified products were digested with restriction enzymes which distinguish methylated from unmethylated sequences as

reported previously (Sadri *et al.*, 1996; Xiong *et al.*, 1997), electrophoresed on 3 % agarose or 5% acrylamide gels, and visualized by ethidium bromide staining.

## 5 RT-PCR

Total RNA was prepared from normal colon epithelium and tumor cell lines using TRIZOL (GIBCO-BRL). To study gene expression following demethylation, cell lines were treated with 1 M of 5-aza-2'-deoxycytidine for 2-5 days. cDNA was prepared using random hexamers and reverse transcriptase as specified by the manufacturer (Boehringer). The expression of versican was determined by RT-PCR using the primers

VF 5'-GCTGCCTATGAAGATGGATTTGAGC-3' (SEQ ID NO:52) and

VR 5'-GGAGTTCCCCCACTGT-TGCCA-3' (SEQ ID NO:53).

15

The PCR products were visualized by ethidium bromide staining. The cDNA samples were also amplified using GAPDH gene, primers

GAPF 5'-CGGAGTCAACGGATTGGTCGTAT-3' (SEQ ID NO:54) and

GAPR 5'-AGCCTTCTCCATGGTGGTGAAGAC-3' (SEQ ID NO:55)

20

as a control for RNA integrity. All reactions were performed using RT (-) controls where the reverse transcriptase enzyme was omitted.

25

## *Chromosomal mapping.*

The chromosomal location of clones that did not correspond to known genes was determined using a human-rodent somatic cell hybrid panel and a

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radiation hybrid panel (Research Genetics). PCR reactions were performed using 30 ng of each of the hybrid panel DNA as a template in a 40  $\mu$ l volume containing 15 pmol of each primer, 0.5 units of Taq DNA polymerase, (GIBCO BRL), 2mM MgCl<sub>2</sub>, BSA and 5% DMSO. First denaturation was  
5 carried out at 95°C for 3 min. Samples were then subjected to 35 cycles of amplification consisting of 25 sec. at 94°C, 1 min at 60 to 68°C and 1.5 min. at 72°C in a thermal cycler (Hybaid). The final extension time was 10 min. Ten  $\mu$ l of the PCR product were electrophoresed in a 2 % agarose and the genotype of each panel was determined. Linkage analysis was performed  
10 using the RH server of Stanford University as described (Stewart *et al.*, 1997).

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention.  
15 Accordingly, the invention is limited only by the following claims.

What is claimed is:

1. A method for identifying a methylated CpG-containing nucleic acid, comprising
  - a) contacting a nucleic acid sample suspected of containing a CpG-containing nucleic acid with a methylation sensitive restriction endonuclease, under conditions and for a time to allow cleavage of the nucleic acid;
  - b) contacting the sample with an isoschizomer of said methylation sensitive restriction endonuclease, wherein said isoschizomer of said methylation sensitive restriction endonuclease cleaves both methylated and unmethylated CpG sites.
  - c) adding oligonucleotides to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotides to the nucleic acid cleaved by said restriction endonuclease; and
  - d) amplifying said cleaved nucleic acid.
2. The method of claim 1, wherein said methylation sensitive restriction endonuclease is SmaI.
3. The method of claim 1, wherein said amplifying is by polymerase chain reaction amplification.

4. The method of claim 3, wherein said amplifying by polymerase chain reaction amplification comprises annealing primers complementary to said oligonucleotide.
5. The method of claim 1, wherein said oligonucleotide comprises a sequence as set forth in a member of the group selected from SEQ ID NO:34 (RXMA24) and SEQ ID NO:35 (RXMA12).
6. The method of claim 1, wherein said oligonucleotide comprises a sequence as set forth in a member of the group selected from SEQ ID NO:36 (RMCA24) and SEQ ID NO:37 (RMCA12).
7. The method of claim 1, further comprising adhering the amplified nucleic acid to a membrane.
8. The method of claim 7, further comprising hybridizing the membrane with a probe of interest.
9. The method of claim 1, wherein the CpG containing nucleic acid comprises a methylated CpG island.
10. The method of claim 9, wherein the CpG island comprises a CpG island located in a gene selected from the group consisting of a p16, a Rb, a VHL, a hMLH1, and a BRCA1 gene.

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11. The method of claim 1, wherein said sample is selected from the group consisting of a brain cell, a colon cell, a urogenital cell, a lung cell, a renal cell, a hematopoietic cell, a breast cell, a thymus cell, a testis cell, an ovarian cell, a uterine cell, an intestinal cell, serum, urine, saliva, cerebrospinal fluid, pleural fluid, ascites fluid, sputum, and stool.
12. The method of claim 1, wherein the presence of methylated CpG - containing nucleic acid in the sample is indicative of a cell proliferative disorder.
13. The method of claim 12, wherein the cell proliferative disorder is selected from the group consisting of colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, uterine cancer, astrocytoma, glioblastoma, and neuroblastoma.
14. The method of claim 1, further comprising performing representation difference analysis, wherein said representation difference analysis comprises hybridizing a driving nucleic acid as a driver.
15. The method of claim 14, wherein said representation difference analysis uses nucleic acid isolated from a member of the group consisting of normal colon, normal lung, normal kidney, normal blood cells, normal breast, normal prostate, normal uterus, normal astrocytes, normal glial and normal neurons.
16. A nucleic acid identified by the method of claim 1.
17. A vector comprising the nucleic acid of claim 16.

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18. A method for detecting an age-associated disorder, associated with methylation of CpG islands, in a nucleic acid sequence of interest in a subject having or at risk of having said disorder, comprising:
  - a) contacting a nucleic acid sample suspected of comprising a CpG-containing nucleic acid with a methylation sensitive restriction endonuclease, under conditions and for a time to allow cleavage of the nucleic acid;
  - b) contacting the sample with an isoschizomer of said methylation sensitive restriction endonuclease, wherein said isoschizomer of said methylation sensitive restriction endonuclease cleaves both methylated and unmethylated CpG-sites, under conditions and for a time to allow cleavage of methylated nucleic acid;
  - c) adding oligonucleotides to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotides to nucleic acid cleaved by said restriction endonuclease;
  - d) amplifying said cleaved nucleic acid;
  - e) adhering the amplified digested nucleic acid to a membrane; and
  - f) hybridizing the membrane with a probe of interest.
19. The method of claim 18, wherein the sample is selected from the group consisting of brain cells, colon cells, urogenital cell, lung cells, renal cells, hematopoietic cells, breast cell, thymus cells, testis cells, ovarian cells, uterine cells, serum, urine, saliva, cerebrospinal fluid, pleural fluid, ascites fluid, sputum, and stool.
20. The method of claim 18, wherein the probe of interest is a nucleic acid sequence.

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21. The method of claim 18, wherein the nucleic acid sequence is selected from the group consisting of a p16, a Rb, a VHL, a hMLH1, and a BRCA1 nucleic acid.
22. The method of claim 21, wherein said nucleic acid sequence is a p16 nucleic acid sequence.
23. The method of claim 18, wherein the sample is a tissue sample or a biological fluid sample.
24. The method of claim 18, wherein the probe is detectably labeled.
25. The method of claim 24, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, and an enzyme.
26. The method of claim 18, wherein said age-associated disorder is selected from the group consisting of atherosclerosis, diabetes melitis, and dementia.
27. The method of claim 18, wherein said age-associated disorder is a cell proliferative disorder.
28. The method of claim 18, wherein the nucleic acid of interest is a member of the group consisting of SEQ ID NOs:1-33 (MINT 1-33).

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29. The method of claim 27, wherein said cell proliferative disorder is selected from the group consisting of colon cancer, lung cancer, renal cancer, leukemia, breast cancer, prostate cancer, uterine cancer, astrocytoma, glioblastoma, and neuroblastoma.
30. The method of claim 18, further comprising performing representation difference analysis, wherein said representation difference analysis comprises hybridizing a driving nucleic acid as a driver.
31. The method of claim 30, wherein said representation difference analysis uses nucleic acid isolated from a member of the group consisting of normal colon, normal lung, normal kidney, normal blood cells, normal breast, normal prostate, normal uterus, normal astrocytes, normal glial and normal neurons.

32. A method for determining the response of a cell to an agent, comprising:
- a) contacting a nucleic acid sample suspected of comprising a CpG-containing nucleic acid from said cell with a methylation sensitive restriction endonuclease, under conditions and for a time to allow cleavage of unmethylated nucleic acid;
  - b) contacting the sample with an isoschizomer of said methylation sensitive restriction endonuclease, wherein said isoschizomer of said methylation sensitive restriction endonuclease cleaves methylated and unmethylated CpG-sites, under conditions and for a time to allow cleavage of methylated nucleic acid;
  - c) adding an oligonucleotide to the nucleic acid sample under conditions and for a time to allow ligation of the oligonucleotide to nucleic acid cleaved by said restriction endonuclease;
  - d) amplifying said cleaved nucleic acid;
  - e) adhering the amplified cleaved nucleic acid to a membrane; and
  - f) hybridizing the membrane with a probe of interest.
33. The method of claim 32, further comprising performing representation difference analysis, wherein said representation difference analysis comprises hybridizing a nucleic acid as a driver.

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34. The method of claim 32, wherein the agent is selected from the group consisting of peptide, peptidomimetic, chemical compound, and a pharmaceutical compound.
35. The method of claim 32, wherein said agent is a chemotherapeutic agent.
36. The method of claim 32, wherein said methylation sensitive restriction endonuclease is *Sma*I.
37. The method of claim 32, wherein said amplifying is by polymerase chain reaction amplification.
38. The method of claim 37, wherein said amplifying by polymerase chain reaction amplification comprises annealing primers complementary to said oligonucleotide.
39. The method of claim 32, wherein said oligonucleotide comprises a sequence as set forth in a member of the group selected from SEQ ID NO:34 (RXMA24) and SEQ ID NO:35 (RXMA12).
40. The method of claim 32, wherein said oligonucleotide comprises a sequence as set forth in a member of the group selected from SEQ ID NO:36 (RMCA24) and SEQ ID NO:37 (RMCA12).

41. The method of claim 32, wherein said cell is selected from the group consisting of a brain cell, a colon cell, an intestinal cell, a urogenital cell, a lung cell, a renal cell, a hematopoietic cell, a breast cell, a thymus cell, a testis cell, an ovarian cell, a uterine cell, an exocrine cell, and an endocrine cell.
42. A kit useful for the detection of a methylated CpG-containing nucleic acid comprising carrier means containing one or more containers comprising a container containing oligonucleotides for ligation to nucleic acid, a second container containing a methylation sensitive restriction endonuclease and a third container containing an isoschizomer of the methylation sensitive restriction endonuclease.
43. The kit of claim 42, wherein said oligonucleotides comprises a sequence as set forth in a member of the group selected from SEQ ID NO:34 (RXMA24) and SEQ ID NO:35 (RXMA12).
44. The kit of claim 42, wherein said oligonucleotide comprises a sequence as set forth in a member of the group selected from SEQ ID NO:36 (RMCA24) and SEQ ID NO:37 (RMCA12).
45. The kit of claim 42, further comprising one or more containers comprising a primer complementary to said oligonucleotide.

46. A kit useful for the detection of a methylated CpG-containing nucleic acid comprising a carrier means containing one or more containers comprising a membrane, wherein said membrane has a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33 (MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, and MINT33 immobilized on said membrane.
47. An isolated nucleic acid comprising a member selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33 (MINT1, MINT2, MINT4, MINT6, MINT8, MINT 9, MINT10, MINT14, MINT15, MINT17, MINT19, MINT20, MINT22, MINT23, MINT24, MINT27, MINT30, MINT31, MINT32, and MINT33), and degenerate variants thereof.
48. The nucleic acid of claim 47, wherein said nucleic acid is methylated.

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49. The nucleic acid of claim 48, wherein said nucleic acid is unmethylated.
50. An substantially purified polypeptide encoded by the nucleic acid of claim 47.
51. The nucleic acid of claim 47, wherein said nucleic acid is operatively linked to an expression control sequence.
52. The nucleic acid of claim 51, wherein the expression control sequence is a promoter.
53. The nucleic acid of claim 52, wherein the promoter is tissue specific.
54. An expression vector containing the nucleic acid of claim 47.
55. The vector of claim 54, wherein the vector is a plasmid.
56. The vector of claim 54, wherein the vector is a viral vector.
57. The vector of claim 56, wherein the viral vector is a retroviral vector.
58. A host cell containing the vector of claim 54.
59. The host cell of claim 58, wherein the cell is a eukaryotic cell.
60. The host cell of claim 58, wherein the cell is a prokaryotic cell.

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61. An isolated nucleic acid sequence comprising a methylated nucleic acid having a sequence as set forth in a member of the group consisting of SEQ ID NOs:1-33.
62. A method of identifying a compound that affects methylation of a nucleic acid, comprising:
  - a) incubating components comprising the compound and a sample comprising a nucleic acid sequence identified by the method of claim 1 under conditions sufficient to allow the components to interact; and
  - b) determining the effect of the compound on expression of the nucleic acid sequence.
63. The method of claim 62, wherein said sample is a cell.
64. The method of claim 62, wherein said sample is a substantially purified nucleic acid.
65. The method of claim 62, wherein the compound is selected from the group consisting of a peptide, a peptidomimetic, a chemical compound, and a pharmaceutical compound.

Figure 1

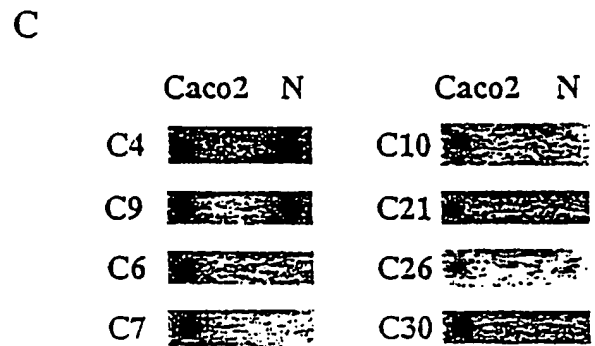
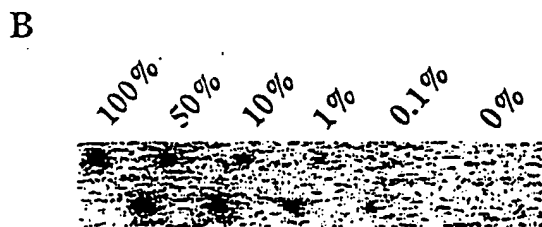
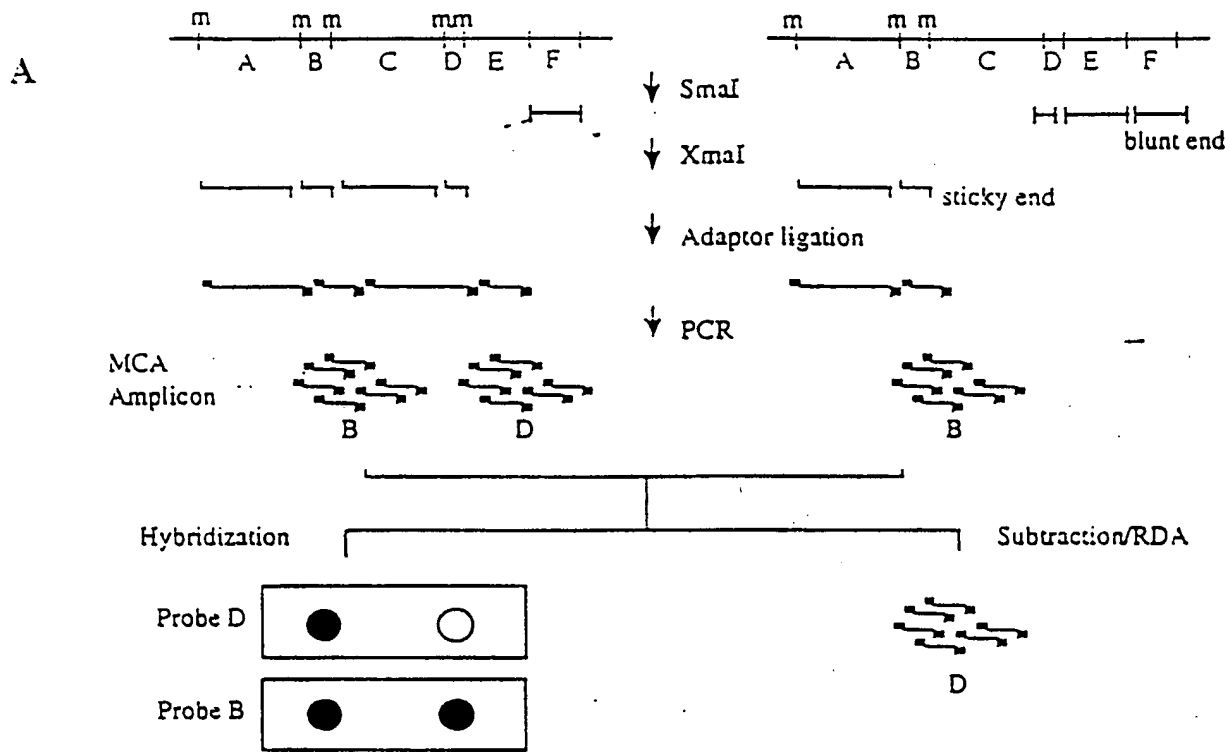
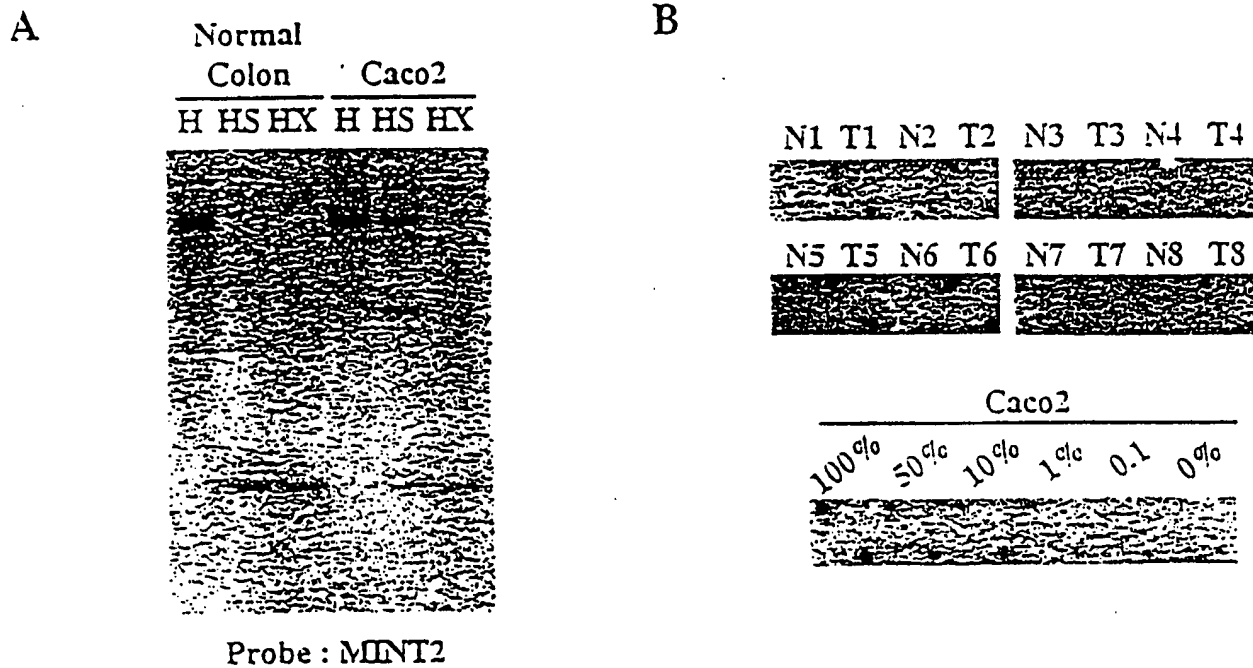


Figure 2



C

SmaI

```

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TCTTTTATTT ATTATTTGT CTCTCCCGCC ACCCGCCCTT TAGTCTTTCC CTCTCTTTAG
TCTTAGTAGC TGCTTTTAAT GGAAGTCSAG GCAGTTGGGT AGTTGGTGCA GCGAGTCCCG
TGCTGCTTTT TATAAATACA GGTAAAATAA TACCCAAATT TCAGGCTGAG GTGCAGTTTC
TTGGAAGAGG AGGAGGGTGT TCCTCTCTCT CCGTCCCTCT TCCCGCTCC CCCGTTTATT
AGAGTATCCT CCGTGGAAAG TGCCAGAAAA ATGTGCTGCA TCTCTGAGTC ACCGTTTCTT
CCGCGCGAAC TCTAGCACCC AAGTTCGCTG GCGGATTTTG GACTGTGCEA AAGTGGTGAG
TTGCTCGTTT ACTTTGAAAG CCTGAAATAT AACTGATGTC CAACTGCAGA AAGGCGCAGC
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```

SmaI

MINT2 : 562 bp

D

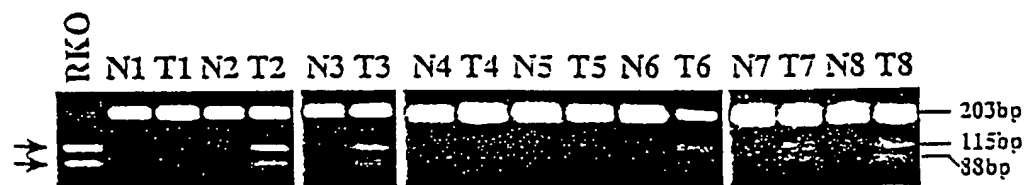
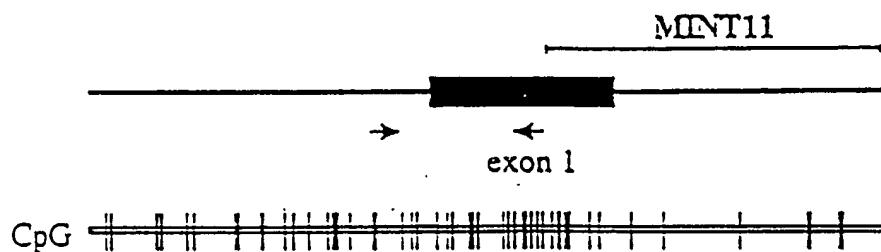
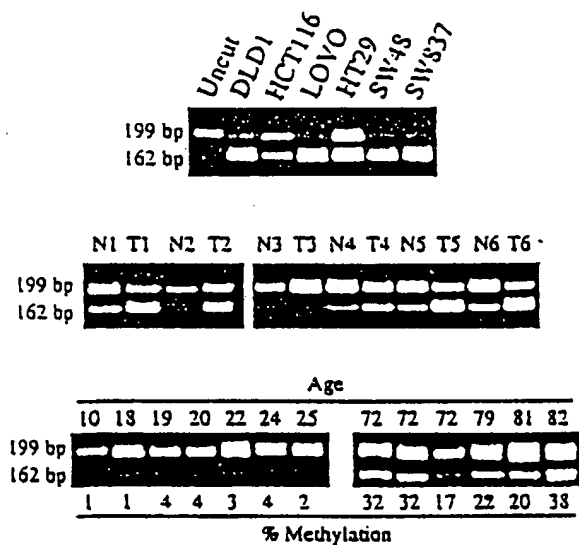


Figure 3

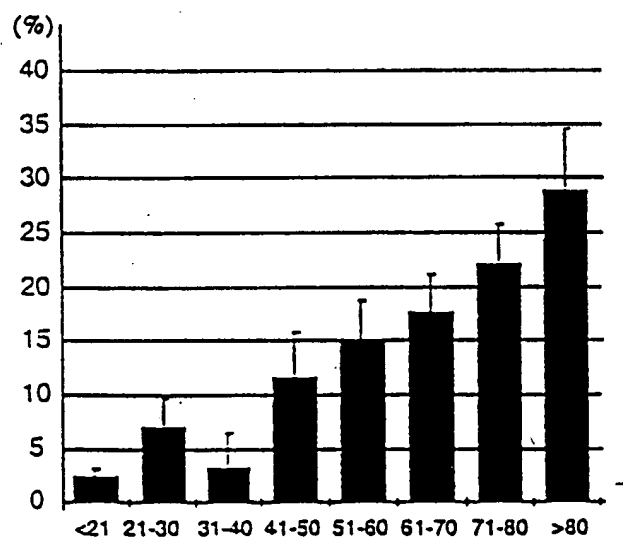
A



B



C



D

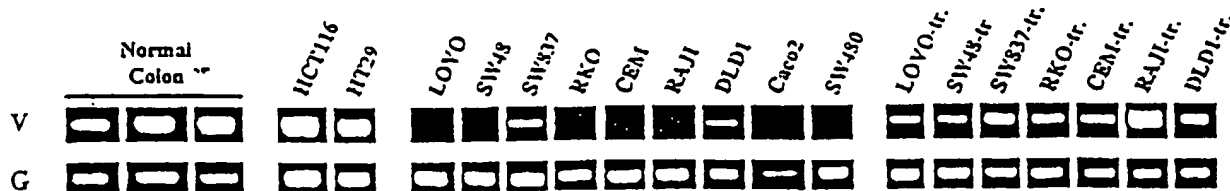


Figure 4

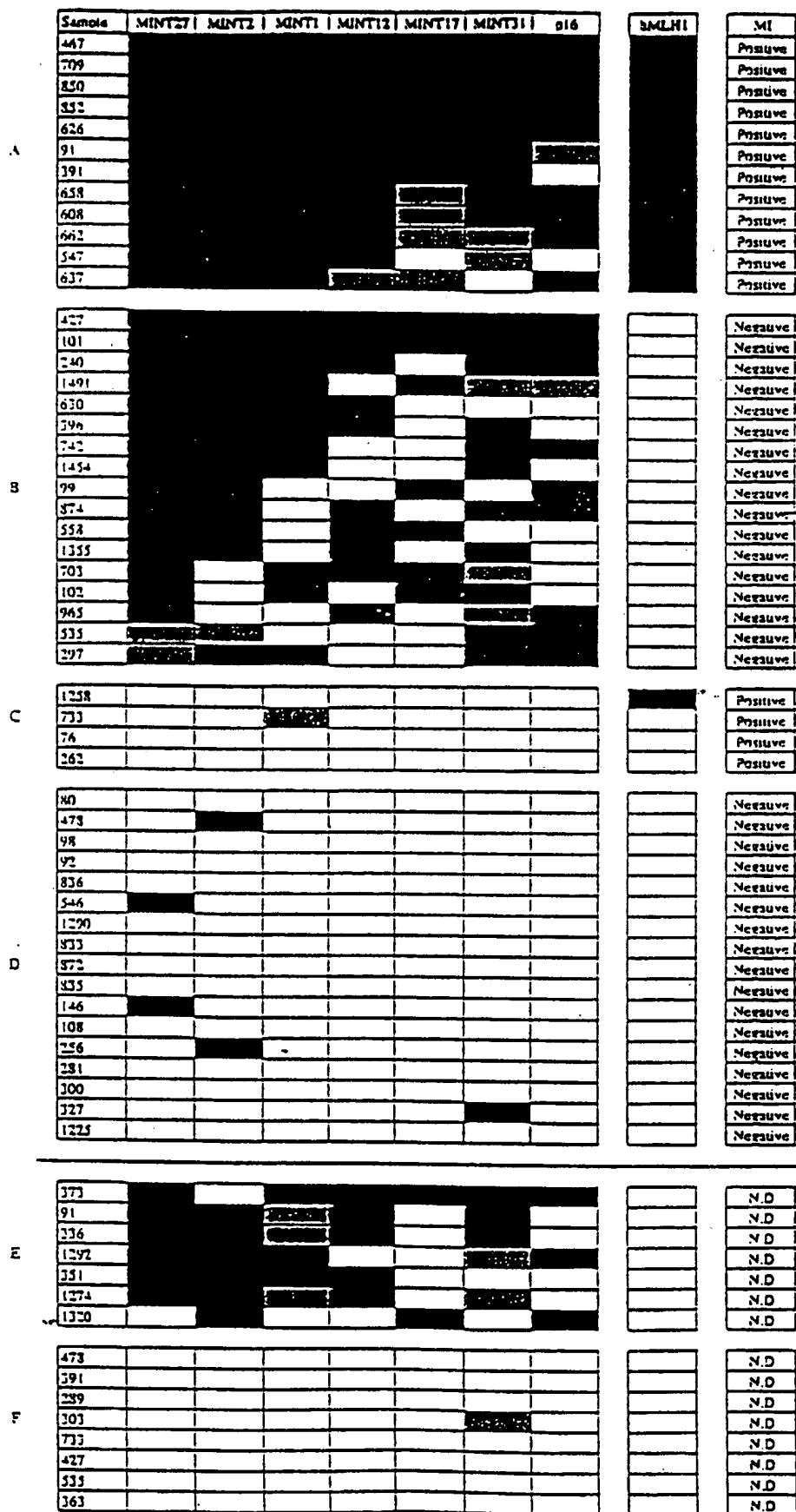


Figure 5

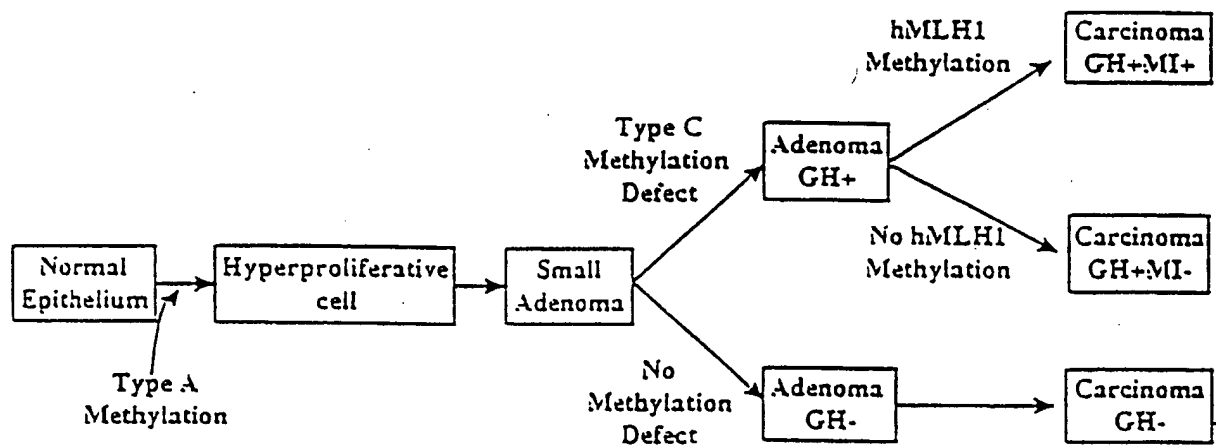


FIGURE 6A

## MINT1 (SEQ ID NO:1)

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GGATCCCTCT	GCAGACGTGC	AGGTGGCGGG	AGAGCAGAGG	TAGCCGCAGT	AAGTGCTGAG
AGAGCCTGAA	AGAAACACCA	TGAATTTTCA	AACTCTCCCA	CATACATTCC	CGAAGCGCCT
GTCTGGCGTC	TAAGAGAGAG	CAAGAGAGGG	CTGGAGAGCA	GGGGAGCCCG	CGGGGCTGAG
GCTCTTTGTC	AGCGCCTGCA	CTTCCTACGT	TACAACGCCT	TCATTTCAGCA	AAAACCTTTT
GGGCGCCTGC	TGTGCGCCAG	GCCAGGCGAA	GNAGACCGAG	GNTGTGAAGC	TCAGAGGGGA
GAGGGACCAA	TCGCAGTAAA	TAAGCTACCG	AGGTAATCTT	AGATGGNGAT	GAGGGCAGGA
AAAGNCATCA	GNCGACCTCT	GACCTTTCTC	TTAGGGGGTT	TTCCCCTTCC	GCCTGGGTTC
TAGAACTGGG	AAGANTTTTC	TCCAGAGCGT	CGCGGGGAGC	GCCCCGGG	

## MINT2 (SEQ ID NO:2)

CCCGGGCGCT	GCCAAATGTA	AACAATCCGC	CATGATTTCT	TTGTTTAGCT	AATCGAACCT
GCCGCCGTCT	CGAGTCCCAG	GCGCTCCCTC	TCCCTTCTCT	CCCCTTCCCC	CCTCGCGCTC
TCTTTTATTT	ATTTATTTGT	CTCTCCCCC	ACCCCGCCCT	TAGTCTTTCC	CTCTCTTTAG
TCTTAGTAGC	TGCTTTTAAT	GGAAGTCGAG	GCAGTTGGGT	AGTTGGTGCA	GGGAGTGCGG
TGGTGGTTTT	TATAAATACA	GGTAAAATAA	TACCCAAATT	TCAGGCTGAG	GTGCAGTTTC
TTGGAAGAGG	AGGAGGGTGT	TCCTCTCCTT	CCCTCCCTCT	TTCCCCCTCC	CCCGTTTATT
AGAGTATCCT	CGGTGGAAAG	TGCCAGAAAA	ATGTGCTGCA	TCTCTGAGTC	ACCTTTTCTT
CCCGCCGAAC	TCTAGCACCC	AAGTTCGCTG	GCGGATTTTG	GACTCTGCCA	AAGTGCTGAG
TTCGTCGTTT	ACTTTGAAAG	CCTGAAATAT	AACTGATGTC	CAACTGCAGA	AAGGCGCACG
GAATCGCCGC	CACCATCCCC	GG			

## MINT3 (SEQ ID NO:3)

CCCGGGTTTC	CAGCTTCTCC	CTTCCACCTT	TGTCTCCCTT	CCCTCCTACA	AACTTTCAGC
CTTCAGTCTG	TTGGGGGNTA	AGATCTGGGA	AATAAGCGTG	TGTGTCCGAG	TGCCTTAGGG
TCTGCTGTGA	GCCTGAATGC	GAGTCCGGTT	GGTTGTGCAG	TTATGAACCT	GTGTGTACAT
CTGTGTGCCT	AAGACGCTGG	GCACTGTATC	CCACATGACC	GATGTGTGTG	AACGACTGTG
TGCCTGTGTG	TCTGGATCTG	CGCGTGGGTG	TAGTTCGTGT	GGCTGTGTAA	ACCGCATGCA
TAGGTATACA	TGTACCTATG	TCTCAGGCTG	TGTGCGTCC	ACTGCGATGG	TACGAGAGTG
TGGGTGTGAT	GGTGTATGTG	ATCCTGTGTC	TGCGTGTCCG	TACATTTGAG	TGGGTGTTGT
GCGTGTGACT	GTGTAAACTG	CGAACATGTA	CGTGTGCCCC	CCCGTAGGTA	TTACCGTGTA
CGTGTGTCTC	CGTGTGCCTG	TGAGGGGTGG	GGTCTGCGCG	GGGATTCCCC	ACCCCCCCAC
ACTCACACCC	TCCAAGCCCC	GGG			

## MINT4 (SEQ ID NO:4)

CCCGGGCCTC	TGGCCCTCTG	CGTCTGCTAG	NCTCTTTCCC	CCAAGACTCC	CCGAGGTGGG
GAGAGNACTG	GTGNTCCCTG	GAGAAATCAA	GGTGTCCAAC	ATTCTCTCCG	AGGCGAGGCT
GCTTGAGCGC	CAGCAACAGG	NCCTGCTGAA	CTTTCTTCCC	CGGCTCCTAC	GCTCCGGTTG
CTCTCCATCC	TCATTTCTGG	GGTCAAATGG	NAAAGAGGGA	ATACTCCTCG	ACCCCTCTCC
CCCTTGACTA	TCCAAAGCAG	CCCGAAGTTG	GCGAGGAGAC	TCTGCCGGGT	GTNCGGGCAA
ATGNCCCCGC	GGGTGGCTCC	AGAAATGGNC	TGTGANCTGC	ACTCGCCTCG	GAGAAATTCC
AACTCTTGGT	TGAAGACTCT	GACTCAGAGG	AGCCCTCTGA	GGATGCGCCC	CTGGAGAAAG
NGCACGGGAG	GGAAAGTGGA	GAGAACTCGN	CCTCCCCAGG	GGCTAGNCAG	CTACTCCCGG

G

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FIGURE 6B

## MINT5 (SEQ ID NO:5)

```

CCCCGGGTAAG TGAGCCCTGC TGCACCTCCG CACCCCTCTT CCCCATGCCC
ACCCTCCGGG GATGCAACAC CCTGTTCCCA TGGAACACGG GGGTTGGCAG
TCACACTGTC CCCACCCAGC TTCAGGCTTG GTCTCCTCTA GGTTCGCTT
CTGAGGAAGC AGTCCCAGGG CATTACTGA CCAANCAGAA AACAGGGGTT
GGGAAAAGTG AGTAGGTGGG TCTGCAACCG TTACAATCAC ATCACTTTAT
TCTTAATTTC AGTATATAAG GATTGGCATC ATANTGGGAT GANGAAGGTT
ACTGTCCTTG TCATTTGGGC AAAAGACCCC TACCCATATC TCAATGACCA
ATTCCTCANA AGTGTCTCTT TGGAANAANC TGANTTTTCC CCTCCGTAAN
TCCTTAACAC TCTCCGGCTT CCCCTCAATC CCAGGCCTTC CCCCTATTGA
CCTCAAGACC TCCTTTAATC CCAAAGAGNC GTTGACTTCC NCCAAATGCG
GGTGCTTTCC TTTCCAATCA AGAATATGTT TAAAAACCCT CCCAGGGAGT
ATGGACTATG TCCCCAATTT TAAAAGATGG AGGAACAAAG GCCCATTGGT
ATGCTAAAAA CCATGGGAAC AGGATCCAGA TTTCCCCCA TCAATTCGAN
CTGCCAGTCT GTCCTCGGAG ATCCTTTGAC TTCTTGGAAT ANCCTTTTTG
TGTTGTGGTT TGGGGGTTGA TCTTGAGAA CTTTTTTGTG TGTCTTTTAA
AAAATGTTTC ATTNGTTAAC TTTCCAAGTG ATGCTCTGAT TGGAGCAATC
TCAACACCAA GAAGAGTAGG GAAAGAAGCA GCGNGGTCC TGGGTCCCCG
GG

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## MINT6 (SEQ ID NO:6)

```

CCCGGGCCCT CAGAGGCCGC ACCACCTATT GTGTTCCAGG CTCGCAGGAA GCCAGACCTT
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GACAGTGGCT GCAAAGGCAA AATCGGGTGT TATTTTCCCA AGAGTCCCTT CAGCGTGAGT
GCCGGGGTCA GCTCGAACTG GAGCCTGTAA TTTGTGAGTG CGAGTGGGGA GCAGCAGGAG
GATCCTTTTC ATAGGTGAGG TCCCAGGAAC GAGCCTGGTC NGTGCTTAGG CAAAGGCCCT
TCCCACCTGT CAGCCTTGTT GTTTACCCAT CCCCTGCTTC TCCCAGACTT GCATTAAATC
CGNNGTCGGT TCCATCAACC CCGGNATCCC CTCCCCCGGG

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## MINT7 (SEQ ID NO:7)

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TTAAGGCCCT GAACACATTG AGCAAAAAGT AGATCTGTCT GTACAGACGT TTCTTTCCAC
GTCTTTTATA GTAAGGACTT TATTA AAAAG CAGGCACTCG AATCCTAGGT GGGTAGATGG
GAGGCTGGGG CTGGGGATGG GGACGTCTTC TGTTTTCTGG TTGTGCACAT TAAAAATAAC
TCTCTGCTGC CTACTCCTAA ACGCAGCCGG CAAAATGAG ACGTCAACTA AGCGCCGTTT
CAGTCCCAGA GCCAGGTCGT CCATGGGGGT TTTCAAGCGT TTTCTCGATG ACTGATTTTT
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G

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## MINT8 (SEQ ID NO:8)

```

CCCGGGCTGT GGCGGTGCAA CTCCCGCCGC CTGCGTTCTA GACAGAAAAG CCCCTTCTGA
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GCCCAAAGGC CCCAGGAGGG GGGTCATGTG CGCCCCAGTC ACTCAGGCTC CCCTCGCTTC
TCCGGTTCGA GTTATGCCAT CAAGCTAATA TATTGTGACT GCTCTTCTCT CCTGTGACAA
AAGGCTTGCA GCTGCCTCCA AATCAATAGA TTGTCAAAGA AATATTGAAA ACAATCATGA
CACAAAACCT TAATCCTGGN TTGGAGGCTA CATAATCAGA AATTGTGCTA CTTGTTCTTC
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TTTTCCAGC AGATCCTGCT ACGTCTGTG GGTGTGTAAT GTAATTTGTA ATTNCTNCCC
TTCATGTGGT CCGGTGCCTT GAACCATCTT TAATTAAAAG CATAATTAAG GGAAGATCTA
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CGGGTCTGT CCCCCGGG

```

FIGURE 6C

## MINT9 (SEQ ID NO:9)

CCCGGGTCAT TGTCCATCTC CGACCAGGGG AGTAGCCACC CCCACTAGCC AGCCGTCTTT  
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 CCAGCCCCAC TACGGAATGG GAGCGCATT TTAGGGTGGTT ACTCTGAAAC AAGGAGGGCC  
 TAGGAATCTA AGAGTGTGAA GAGTAGAGAG GAAGTACCTC TACCCACCAG CCCACCCAGT  
 CCCTCTCTCA GCAGTGGATG GGGATGGGGT GGGGGTAGGG ACGAGAAGGC AGCTGGTGGA  
 GAAACAGCTT CAAGACTCTT TGGGTTCCTC CTGCTCTCCA GGGGAGCTTA CCTGGGGCTA  
 ACTTTAGACA CACAGGTTTG GAGGGAGGAA AGAAGGAAGA ATTCTTTTAC AACGAATCAA  
 TTAAGAGCAC TTCCTCTTTT CTTAACTTGG GGGGAGGGCC AGGAAAACCTT CTTGAGTCAA  
 GAAATCTTGG GAGGNAGACA TCAGATGTNG GCAAGAGGCA GACAGATTTT GGAAGGCAG  
 GCTTTGGGTC AANAAAGATC AAGCCTGTGT TGTCCCAGG CTCTTCCCTG TTCCCCATC  
 CCGGG

## MINT10 (SEQ ID NO:10)

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 CCTGAATGTG ACNGCTGCGT CTTCTGCCA CACCTACTTT TGAATGCCAA GAGGGGGCTC  
 CGCTGGCCAG GACNGAATAT TTTTATGGTA AAAAATGACC GGCAGTTGCA TCAGCTCCAG  
 GAGGGTGGGA GCCGTCACCC GAGGTGCGAC AGGCAGACTG ATGAAAATTC TGCTTATAAA  
 GTCACTGCTC CCCCATTAAT TAGGGGGGAG GGGGCGCTCC GGAGCCACCA CGCACCTCGC  
 CCACGNCNA AAAGCTTGTC AACATTTTCC ACGAAGGATT GAAAATGTAA ATTAACTTTC  
 AGATTATTCA ATGTCACCAA GGTATGGAAA AAGGTCGCCA TACTGGGTGT CATTTATCTC  
 GTTGTGGATT TAAAGAGCTT TTTCTATTAA ATTTCTTAAA ATTAATGTTT TATGTTGCTC  
 AGAGTAATTT AAACAATTAT GGGCTTAAAG AATTGATCAT TACAGCCCCT GGGATTTAGC  
 GCTGCAGGCT GATNNCCCTG AAAAACCTCT GATTTATCAG GGNTCGTATT NGGCCGGGCA  
 AGCCCGGG

## MINT11 (SEQ ID NO:11)

CCCGGGAGTG GCTAACCAGG AANANNAGGC ACTGNCCACA CACCANGGGC TGGGAAATCA  
 AGTGGCCTGC ACCAAGGCGG CTTCCGGGGG CTTGTCTGTG GCAAGTCTTG GTAGTCCCCA  
 TTCAAACCTT TGCCTCGAGC GTGTTAAGAA CAACAACAAA AAAAAATCA AAGTGCCAAA  
 GGTCCCTCTC TTCTCTCCAG CTCAAGAACC CACCACTTTT CTATGATTTT TTTACAATTT  
 ATTCCCTCCC TTCCCCCAAT TCCGTTAGTC ACTTTACCCC CACCCACCCC TGGGTTTCTT  
 TTGTCTGAAT CTTTTTCAAC ACCAAGGTCC CTCTGTATGC CTCTCCCCAA AAGCCCTTAT  
 GAAAAGTTAC CTGCATTTTT TAAGTGCCTA CATTTCTTAA CTTGCGCTAA CAGCTCTTTG  
 CCTTAATTAA AGCCTTCTAC CAATTGCTTC TTTTTTCTAA GCTCGCGGGT TTTTTTCAAT  
 AAGTTTTTTG TTTTGTGTTT TTAAGGGGGG AACAAAAGAA ACGTGATTAC CTTGGAAGGC  
 GGCTTATTGC AGTTTGGGGG GAAAATTCAC TGCAGCGCTG CGCGACTGGG TTCGGCGTTG  
 CCCAGGCGGG TCACATAGGA AGCGTGGTGG CCCGGG

## MINT12 (SEQ ID NO:12)

CCCGGGTCCC AGCCCTGAGG ACCAGGTTTC AGGGCTCAGA AGACTCCAGC  
 GAGGTTCCCT CGCAGATTGT GTCTGCGGTC GTTGGGGGAG GGGCCCCGCA  
 GCCTTCAGCA GATTATCCAA AGGTCAGTGA CCCAGATATG GTTTTGGNCA  
 CGGGCCATGT TTTACTTCCT GTGCCCCAAG CAGAATTTAG CTGAATAATT  
 CGGACCCCAA ACCAAACAAA ACGTCTTTAT TTCCGTTTGG GGATTCTTCG  
 GAGTTGGGAT TTTTCTGTCT CAAATTAGAA TAATNTGCAT TATTAACCAA  
 CTTAACATTT ATTTGTTGGT TGGCTGCCTG ACCTTTCTGA GCCTCAGTTT  
 NTTGCTCTGT AAATTGGGAG CTTACCCAGG TCGGAGGACT GTTGAATTG  
 GAAATATTCT AATAAGGAAG TGTTTTTGCA AGTGCTTTGT AAGCAGCAAA  
 GCGCTTCTTC AGGGGTCAAT TTTTTTTAGC TCTGCAGTCA CCACCCAAAT  
 TCGGAAGATC GTTGTGCCTT TCTTGGATGA GAATGCCCGG CTCCAGCCCG  
 GG

FIGURE 6D

## MINT13 (SEQ ID NO:13)

```

CCCGGGTGTG TGTGTGTTCCC CTCCATGTAC CACGTGTTTG TCCTGATGGT CTCCTACCCC
CTGTCCCCCT GAGAGGCCCT GGTGTGTGTT GTTCCCCTCC ATGTATCCAC GTGTTTGTCC
TGATGGTCTC CTACCCCCCG TCCCCCTGAG AGGCCCTGGT GTGTGTTGTT CCCCTCCATG
TACCCACGTG TTTGTCTCTGA TGCTCTCCTA CCCCCTGTCC CCCTGAGAGG CCTGGTGTG
TGTTGTTCCC CTCCATGTAC CCACGTGTTT GTCCTGATGC TCTCCTACCC CCTGTCCCCC
TGANANGCCC GGG

```

## MINT14 (SEQ ID NO:14)

```

CCCGGGAGTG GCCCTGCCTG GCCATTTGCT CAAGCAGCAT GCAAGCCGGA TCTACAGCCG
GTGCACCTTG TTCCTTGTTT TCCCGGGCAC CGGAGGCCCA CGTGAAACCT CTCAGAGGAG
AGCGAAGAAG GCCACCTTTC ATCTCAATGA GCCAACAGCC TCACATCCTT AAGTCCTGCC
TAATCTTACG AGTCATGAGT CATCGCTTCT TCCCCGCCAA GTCATTGAGG ATTCAGTGAC
TCCAGCTGCC CTCAGGATCT GACGGAATCT TGAAGGCAGA GTTCCGAGAC TGCAGTCCAG
GATAGAGAAG CCCCCGGCTC CATCAGGGGC TCCTCGGCTT CAAGGCAGGA CCCACCCCAA
AGCTNTCAA GCGGCAGAGG CCTGTTTTCA GGTCTATTTT TAAAGATNTC TAGGGGAAGT
GGTTTCTGAA TGCTCTGTAG GATGAGGCTG TGAACAGTGA TTGTTTCATT TGCTCGGGGC
TGGCAAAAAA GGGATGTACA ACCCGTTCTG ACCACACCAG TGAGTTAAAA AGCACTGCAG
ACTATGAATA CCTTTTGCCA GTTGGACTGG TTATGGGGAC CGATGGCTTC CCCTACAAC
GGGGAGGCTG CCAGCCCGGG

```

## MINT15 (SEQ ID NO:15)

```

CCCGGGACCT CTGCGGTTAC CTGGGCCTTC CCTGCCAGCC CCCACCCCCT GCCCCACCA
GAAAGCGTTT ACAGGACAGG TGAGGCCCCG AGGAGGAAAA GCACTCCCCT GCGCAACAT
GACTCCAGCG CATCTGCGTC TAAGCCACAC CGTGCTCCTG GTAGATTAAA AATTAATTCT
AAAAAAAAAA TCTCTCCTAT CCCAAATGCA CTGTTTTCTG CCTTGCTTGA CAATTGATTT
GTTTTTAAAG GAAAGTTATG GGTAGATCCT CTTTTTCTT TCCCATTCTT TNNTTCTTCT
TTTATACTGG AGGGAGGGAA ACGGAGGCGA GGACACACAC GCGCAGGCAG GGGNTGAAAA
GGCCGAGGTG GGTTTTCCTG TTTAATATCA AAGGAGGGCG AATAATGGGT TTCCTCGGTC
CGGCTAGGCC GGCCTTTGAC TCAATTGGAA ATGCAAAGGC AGCTTTTGCC TATTNTCTGG
CTGCTGGCTG AGACCCTAAA TTTCCGTAGG AAATCGTCGG ACACGCACTT AATCGGNCTT
TGCAANCTTT CCCTCGAAGT TGCACGCGGG TCTGGGCGGA GGAGGCGAGG TAACCCTGGA
TTCGAACCAG CGCCTTTCTC TCCTTCAGGC CTCCGCCCCG G

```

## MINT16 (SEQ ID NO:16)

```

CCCGGGACAA GCGGGGTCAC CTCTGGGGCC TCACCGCAGT TCCACTTCCT TTCTCGGGTA
TTTGGAACC GTACCCCCGC CATTTGCGTG TGGGAAGAGC GCGCGGGCCC TGCCGGACTT
TAGTGCTTTA GGGGTAAATT TCGGGCTGAC AGGGACGGAG CCTAAGGCAG TGAGCGCCCC
AGTACCCTCA AACCTTATTG CTGGCCCCTG CTGTCTGAGC TTACAAGCAT TACCGCCGCT
ATTTCCGTGC GGGCTGACAC GGGAGATGAA AGTGGTGAAG ACACCCAGGG TGCGGGGGTG
GAGGTGGGGA GAGGAGCCAG ATGGGATTGA TCCCCAGAGC CAGATGGGAT TTAAAGGTGA
GGGAGGAGGG CATCCTGATG GCGTGTGGTC AGTTGATGCC AGATTGGATG GCTGAGACAC
CTCTGCAGCT TACAGGAAAG ACAGAGGGAA AGGGTTCTAT GAATTCTAGC TGTTCTACT
CAAAGCAAAT AATTAATCAA GTGGGGGGGG GGCCTCTAGC TGTAACCCCA TACCTCTAGG
AAACCTTTTG TCATGTGGAG CCACAGTGCT CACTTGACAG ATTCCCCACT GAGAAGTGGG
CTAAGAGGTT GGCCTGCATT GCTGGGTGCC TCCAGGTGGG GAGTCCTGTA CCTGGGAGCC
CGGG

```

FIGURE 6E

## MINT17 (SEQ ID NO:17)

```

CCCGGGCGCC GGGGGTCCCA TCCACTTCTC CCTACCTTCT CTCTCTCCTG TTGGAGAGGC
CAGGGGCTCC CGCGACTGAA AGGGGCCAGG CTGAGGCTGC CCAATCCCAG GGACAGGAAG
AACATTTTGG ATGGATCGCG GGGTGCAGAA AAAGGAAGTT GAGCGACAGA CGCNAAGCCC
AAAGCGTGGA ATTTGGGAAG AGGCAGAAAA AAAGTGGGCG AAGAGGAAGA GAAAAAACNA
GCNACGAGAT GGGGAGGGAC AAGAAGTTTG GGGAGGATAG AGAAAAAGAG AAAGTGCCTG
GAAAACAAGT TGCAAGACAA TTGAAAAGTT GAATGAGAAG GAAAGANAGG AAGGTNGTNA
AATCAAATTA GAACTGTTGG AAAAGANGGC TGGGACACGG CTTTCTCTGG TTCTGTCTCTC
CCAAGAAATT GGAAACCTCT CCCCTTCCCG GCACCAANCT TNCGGGATGT TCCGGTGCCC
CTCCCCGGG

```

## MINT18 (SEQ ID NO:18)

```

CCCGGGAGTG CTCCTGCCGG CAGCATGTCC ACTTGCTAGG GGCAGAGGGG CAGTGGGAGT
GTCCCCCATG TGCCAGCCTG TCCCCACACT TGGGTAAACC TTCAGTCACC AGGAGGAATA
AGGTGTGACA CCTCTGAGAC TGTGGGCACA TGTGCTGCTG CCTGACAGTC ACAGGCTGTA
CTGTGCCAGT GACAGAGGCC ATGATGCCCA CATAACCCAA ACCCGAAGCC GAACCCTAAC
CCCTAACCCC TAACCCTAAC CCTACCCTAA CCCTAACCCA GCCCTAACTC TAGCCCTAGC
CCTAGCCCTA GCCCTAAGCC CTAAGCCCTT AAGCCTAACC CCAAACCCCC AACCCCAACC
CCNAACCCTA ACCCTTAACC CTTCCCTCAA GCCTCTCNA A CCTGCTTGG GTTTACAAGG
TTATTNAACC CCGGG

```

## MINT19 (SEQ ID NO:19)

```

CCCGGGATTG GTCTTTTGGC TGGGATGTAA AGGAGGAGGA ACTAGCTGGG GAAAGGCTGG
GTAAGGGGGA AAACCCAGGG AATTAAACCC CCTCTTCTGT AAAATGAGAA CCCATTCAAT
CACTTAGCAA ATCTTTATGG AGCTCTGTAG GGGCCCTGGG GGAAGGCGAC CAGAGTGTCT
GAAAGCAAAG AGCAGGAGAG TGTGGACTCA GCTGAGAGGA GAGCAGAGGC TGAGTGTGAG
GGTGGGGAGT GGAAGGGGT TTCCAGGTGC CAACAAGGAC ACAAGCCAAG GTGTTGCAGT
AGGGCATTG GGAACGTAT GAGAAGCGCT CCTGCCAGCT TCCCTGGCGG TGTCTTATTC
CCTGCATCCA TTTTGACCA TGAGCCCTT CTCTTACCCT CTGGCCAGGA CCGAATGCCA
TAGACTTCCC CAGATTGCCC GGG

```

## MINT20 (SEQ ID NO:20)

```

CCCGGGCCCC AGGGCGGCCC GAACCCAGC CAAGCCGGCC AGCAGCAGGG CCAACAGAAG
CAGAAGCGCC ACCGGACGCG CTTACCCCC GCACAGCTCA ACGAGTTGGA GAGGAGCTTC
GCCAAGACTC ACTACCCCGA CATCTTTATG CGTGAGGAGC TGGCACTGCG TATCGGGCTG
ACCGAGTCCC GAGTGCAGGT ACGAGGGGCT TGGGATCTGG GACAGAAGGC AAGGACAGGG
CGGGAGGATT TGGGCAAAGG GAGCAGGGTC TTCCCTTCCC CTGTCGAGAT CCTGGGCTGC
TTTCAGGCTG CCTGTGCGTT CCTGTATCGA GTTATCTCCA TCTCTACCCG GAAACTGGTC
CCCATCGCCA TCCCCCAATG GACACGCAAG GCCCGTCTCC GGCCAGTATA GCGACATCCC
GGAAGAAGCT CCTCAAAATC GAAGCCCGGC GTTGTGCGGC TACAGGGCTC GCCTCCTCCG
CCTGAGAAGG CAACCTCAGC GCCCCCGGG

```

## MINT21 (SEQ ID NO:21)

```

CCCGGGAACCT ACCTAACGCT AGTTCAGTCC CAAAATGCTG CCCAACGACA GAATGCTCGC
CTCCTTGCTT CCTCTAACAC TCTGGCACAC CCACTTGGTG TCGGGCCTCT ATGGGCTCGC
AGTGAAGCCC TGAGCCTGGG CTGCCCCCTT CCATGTGCCC CCTGCCAGCC GGCCCTCCCT
CCCTTTGGGT GCCCATCCC TCCAGTCAAC TCCTAGCCGA CCCTTAAGAG TCAGGTATTT
GTAGCCTTCC CTGACATCCC TCCCAGGCTG TCCCAGTGC AGCAGGACGA GCCTGCCCCCT
CCTCCACCCT CTTACAGCT ATACCTAGCC TTGGCCATAA TCACTAATGG ACCAGGAAAC
ACCCTGGCGC GCAGAGCCAC CGCAAAGTGG CCCGCTCAGG CCCGCCCCGG

```

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FIGURE 6F

## MINT22 (SEQ ID NO:22)

```

CCCGGGGATC TCAGAAAGGG AAGGATGTGG GGAGAGTGAA GGTGGAGGCA GTCACACCTA
TCCTGGTGAC CTTGGCGTGC CCCCCTGGAG TGACGAGCCA GGGGCTTATA TAGGTGCGAC
TAGGATGTTG CCTCGTTTTT CACTCGGGGC TGCGAAGAGG GCATTGCTCT TTCTGATGGC
TGGAAGACAG GGGCAAGGAG AGAGAGAACC GGCCCCGAGA CGGGCTGGAG GGTGGGGACA
CTGGGGAGTT TGGAGCTGGG GGTTCGGAGT GGGAGGTTTG GGTCTTCTGA GACGCTCCAG
ACTCTCCGGA GGCGGCAGAG GTCGAGGCAG GAGGCGAATG TGACGCTTAG GGTGCTACG
GTTGATGTTG GGCGCCTTTG GAAGCTGGTC ATTAATTCTT GTCATCGGGA GGTTCGCGG
ANGGCGACAG CGCCCCGGG

```

## MINT23 (SEQ ID NO:23)

```

CCCGGGCGCC CGGCCCTGGC TCGCGGAATG GGCGGCCAGA TCTCAGGCCC TGCCTGCCCG
AGCTCAGTCC CAGTTCCAAC CGGGGGTGCC CATGGACTCT CGGAGGGCAC TCCTGGGGGG
ACAGCTAAGA CACCAGGCTG CAGGATCACT CATTGCACGC TGCATAATCG CCGCCACAAA
CTCTCCCGTG CGCAAGAAC AACGCGCGTG GGACAGAAAA AGTTCCTAGG TCTCCGCAGG
AGTGAATGCA AAATCCAGGG GACTCAGGGT CATGTTGGGA GCCCCTTCTC CCCCCGAGAG
TCAGGGAGCT GTTGAGGTGG GATCGGTGAG GGTGCGGCCA CGCGGGTCCC TTCCCTACCA
GGCTCGGATA CCATGCAGCG TGGACACTCC CGAGTTGCTC TCGGGAATCC CGGG

```

## MINT24 (SEQ ID NO:24)

```

CCCGGGGACG GGGAGGGAGG AGGGCTGCCG GGATGTGAAC CGGGGAAGGC AGCTGGGGCT
GGAGAGCAGC GCGGAAAGGG GGCCCAGGGA GCTGGAAAGC GAGCCAAGAG GAGGGCAAGG
AAGGTGGCGG GCTACGGGGA GGGGAAAGAA AAAGGGTGTC TTGGCGGTGG CCTTGGTAAG
AGAAAGGGGC AAGGGGTATA ATTGACAAGG CACTGAAAGT ATTGAAGTCA GAGCCTTGGG
AAGGATCTAC CGAACTCTCG GCGGTCCACG CGGGGACAGA CCTCAGCCCC TGAGCCTTGA
GCTCCACGCG GGGACAGACC TCAGCCCCTG AGCCTTGAGC TCCACGCGGG GACAGACCTC
AGCCCGTGAG CCTTGAGCTC CACGCGGGGA CAGACCTCAG CCCGTGAGCC TTGANCCAG
AAGGAGTGGC AACCTCANGA CGTTTGCCAA GTGGCCTGGA ATGTTANGGA AACCCAGCC
CCGCCAGGAA CANANCTGGC ACTAATTCCC NGCTCGGNCC GGG

```

## MINT25 (SEQ ID NO:25)

```

CCCGGGGTGG GAGCTGGCTC GTGGGGGCGT GCGCTGCGCG AAAGCGAAAG CCGCCCGCCA
GAGCAACTTT GCGGCGGAAG GCGCCGACGA GGAGCTGTGC CGTGCCGCTC TTGGGGATGG
TGAGCTGGCC GCCCGGCCGG GTGGGCAGCG CGTCCGGGCG CGGTGCTTCG CTAGCTATAA
ATAGGTGCTG TGCGGGGACA GGAAGATGGT TCCGGCCCTT TACAAGCACC GGCCCGTTAT
GTGCGCTGGG CTAGGACCTT GCCCCGCAGC GGAGTGGGAG GAGTGAGGTT AGGGGTAACG
GTTGCATGGG ATGGGGGGTG GGCACATAGA GCCTACAGCA GAGTTGGCGG CGGGGCTCTC
CCATGCACTT GGTGTTTGT CGTTTCTGCT TTTCCCGG

```

## MINT26 (SEQ ID NO:26)

```

CCCGGGCTCC GGCATAGCTC TAGATTAACG AGCTGGGCGA CGGGGGCGGG GGCAGCATGC
CCAGCGTCGG TGCACGGCCG GGGTTCTTAG ACATCACAAA CTGTGGAGCG ATACATTGGA
AGCGAAATCC AAGAACGACA CCGGCCGGCG TGTTTCTGAT GTAGTCGTGA TTAGTGTTGG
AGATGGCCAA GGGCGGCTTG CGGAGCCCAG GGAACGCAGC GAGCCAGGCC CGCGCCCCC
TGCAAAGCTC TGCCTTGAAC CACGTAATTC AGGCACCCAG GGTGTCCCTC CCTAGGTCCT
GGCCACATTT CCCGAAGGTT ATTAAATGGA GAATTCAGCA GTGGAGTTAG AGACGGACGA
ATGTACAGGA AGATAAGAGG GAAACTCTTC CTCATTGCT TTAGGGGGTT GTGCTGGGAA
AATGCGGGGA GTTAAACAA AGCTTCTCCT ANGACTCCAC GATTCAATTC TAACAACCTC
TTAAAAGACT CCCGTCTCNG GAGCAGACGC NCCCTCCCCG CTCTCTAAGC CCCGCTGCAT
GAAANGATGC CCTCGGCCCC TTGCCAGAGG GCCGGGTCCG GGATTCCCGG G

```

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FIGURE 6G

## MINT27 (SEQ ID NO:27)

CCCGGGATCC GGGGAAGGCTC CCCCAGAGCCG GGGTCGGAAC TCGGGCTGGA GGGGCCTCGG  
 CTTGAGGAGG ATCTGGGAGG GCGGGGGCTC AGTCCTGGCC ACCAGGTGTG AGGGGTCTGG  
 TCGGGAGCCC TGTGTCAGAC GCGGCGGTGA AGGCTGTAGC CCTGCTCTCC GGGATGGGGG  
 TGGTACTCTC ACCGCACCCT GCCCAAGCC GCAGGGAGCC CCTGGCGCCC CTGGCGCCCC  
 GG

## MINT28 (SEQ ID NO:28)

CCCGGGCCCC TGGCGGGGAC ACCGGGGGGG CGCCGGGGGC CTCCCACTTA TTCTACACCT  
 CTCATGTCTC TTCACCGTGC CAGACTAGAG TCAAGCTCAA CAGGGTCTTC TTTCCCCGCT  
 GATTCCGCCA AGCCCGTTCC CTTGGCTGTG GTTTCGCTGG ATAGTAGGTA GGGACAGTGG  
 GAATCTCGTT CATCCATTCA TGC GCGTCAC TAATTAGATG ACGAGGCATT TGGCTACCTT  
 AAGAGAGTCA TAGTTACTCC CGCCGTTTAC CCGCGCTTCA TTGAATTTCT TCACTTTGAC  
 ATTCAGAGCA CTGGGCAGAA ATCACATCGC GTCAACACCC GCCGCGGGCC TTCGCGATGC  
 TTTGTTTTAA TTAAACAGTC GGATTCCCCCT GGTCCGCACC AGTTCTAAGT CGGCTGCTAG  
 GCGCCGGCCG ANGCGAAGCG CCGCGCGGAA CCGCGGGCCC GGG

## MINT29 (SEQ ID NO:29)

CCCGGGAGCT GACCGTGGGG AGGCCGGTTC CGCTGGTTTC AACCAGCCCA CTCTCCCCTC  
 TTGGGATGCC CAACCCCGCG TACTCACCAT TTCCTGGTTT CCAGGATGTC CTGGCATCTT  
 AGCTATGCAT TTCCAGTACC TCCAGACCTC AGGGCAACAA AGGATGTGAC AAAGTCACCT  
 AGTGCTCCTG AGGGGCAACA CGCGGGACAG TAGAGATGGA TCTCAGGCTC CGGCCTGAGC  
 CAGAGACAAA GGCCGCGCCA AACGCTGGAA GCCACGCCCT CCTCCCCAAC TCGGTGCCTG  
 ATAGGACGGT TCCTACTCTG ACAGATTGAA TAAGGCTCCA GGACCCCTCC CCACACCCAC  
 CGTCCCCAGC ATTAGTGCGC TTTATGGACA GGGAAACGGG ATCCTGTANG CGGGGTCA  
 CGCCCCGGG

## MINT30 (SEQ ID NO:30)

CCCGGGCACC TGGGCTGGGG GGGCACTCAC ATGGCTACCG GAGGCCCCCA CGTGCGGCGC  
 CCCGCGGAGA CAGGGGTTCG CGTTCAGAGC TGGTGGCGGA TGGACCAGGT GGCCGCGGGG  
 ACCAGCTGGG TCCAGATGTG CTGGGCCTGC TGGGAAGGGGA CAGGTGCTAC CTGGACGTGT  
 AATGGCCCTT GGTCTCTTTT GGCCGAACCT GCCGCTCCGA TCCCCCTCCA TCCCTTCATC  
 CCTCCATCCC TCCATCCCTC CATCCCTCCG GCCCTTCTCC CTTTCTTCCT CCGCTGCCTG  
 TGTTGCAGAG AGGGGCTGTC AGAGACTGTT GATGTGGGAA AAAATGAAAT GGGGGAGGGG  
 TTGTGATTGG CAAAGGCCAG TTGTGCCGGG AGCGGTGGGT AGAGGGGGTG CCCTGAGAGT  
 GGGAAGCCCT AAAGTTGGAG GGCAGCGCTG ATGGGGAGAG GGTTCCTGGC ACCCCACCT  
 GCCTTGGAAG TGGGAAATGA CATAGCGGGA GGGGGGCTGC AGTTCCAGCC CCCGGG

## MINT31 (SEQ ID NO:31)

CCCGGGGCCT CTATCCTGGC GGGGAAGGGCA GGCCGACCCG GCAGACTGCG GCCTCTCGGG  
 AGGGAAGAAG GTGTCAGACG CGCGGAGCAA CCATAAATAG CCCCCCTTC CCAGAAGACG  
 GCACGGGGTT CAAGACTCAG GCGCCGCATA CTCAGAAATGA GAGCAGAGAC TCCCGCCAGG  
 AAAAAAGGGC ACTTAGGGGA TCTGCTCATT AACATGAAAT GCAAATGAGC CCGCCCGGCC  
 TCATTTACAC AACTCTGTGC ATGGATTCCG CGAAAGGGCA ACCAGGGAGA CGACGGCGCA  
 GCAGCCACTC TGCCACTTCC CCCATCCCCT CCCCCCATC GGCCGGGGCG GGAAGTGA  
 CGACCCCAAC CCTCTGCGGC GCGGGGAGGT GCGCGGGGGC TCGGTGGGTG GTGCAGCCTT  
 AGGGGAGTGA ACAACGCCCA GGGGTGATGG CCTCAGCAA GTGAGGGGTG GTGATGGAGG  
 TCATCCGACC CATCCGCGG CCTCTCCGCA GTGGCGCAAG CGCCCCAAA TCTCCGGAGA  
 NGGAAGTGA TGACCCACTA GGTTCGCGCG TGTCTACCTC TCGCAGATGT TGGGGAAGTG  
 CTTCCCGGCG TCTAATCCTC GCTGTTCCCC CCTCCACCGG CGCCAGCAC ACCCGCGGCG  
 CTCGCTCCC GGG

FIGURE 6H

## MINT32 (SEQ ID NO:32)

CCCGGGTACC	TGCACAGCTC	GCTCCCTCCC	ATCCTTCGGG	TCTTCGCTCG	AACGTCCGCT
CCTCGGTGAG	GCCTTCCCTG	GACAACGCAT	TTGAAACGTA	ACCCCAAGGC	AAGAAGCCAC
CTTCCAGGCG	CGCAGCCGAA	GCCCAGTGCC	AAGGAGGCCG	GAGACTCGGG	TGCCCCGCGCA
TCCCGAAAAC	AGCCTCTGAG	GGGTCCTCTG	AGCATCCTTC	CAGCGTGTTT	GGGAGGCAAA
CTCGTTGACT	AGCTCTTGAG	AGGAGTGGCT	AGAGGAATCC	AGGCGGGGAA	GGGGACGGTG
GACTCCAGGA	GAGTGTAATT	TACAAAGGCG	GGGGGCGGGG	ACGCCCAGGT	CCGAGTCCCA
GGACTCTGCG	CCGGACGCTT	CGCCCGCCCT	TTCAGGTCCC	CTGCCCCGGTC	CTCGTACCCG
CGCGGGTCCG	GAGAACCTCT	GAGCACCGGC	CCCCAGCCCC	CGGG	

## MINT33 (SEQ ID NO:33)

CCCGGGCAGA	AAGGCTCGGA	TGGCGGTGGC	AGAAAGGCTC	GGAGGCGGTG	GCCTCAGATC
CCTGGTCCCT	CCGGGTCACT	GTCGGCTAAT	TCTGGGGGAA	GGACTGGGCA	AGGCTGTTTG
GAAGAAGTCA	GCGCCCGGG				

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/25251**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 15, 19, 91.2, 91.52, 199, 320.1, 810; 536/23.1, 24.31, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97/45560 A1 (NORTH SHORE UNIVERSITY HOSPITAL RESEARCH CORPORATION) 04 December 1997 (04.12.97), see entire reference, especially pages 2-6, 14-15, 23-24, 28-29, 35.	1-4, 7-27, 29-38, 41-42, 45, 62-65
Y	ZRIHAN-LICHT et al. DNA methylation status of the Muc1 gene coding for a breast-cancer-associated protein. Int. J. Cancer. July 1995, Vol. 62, No. 3, pages 245-251, especially pages 246-259.	1-4, 7-27, 29-38, 41-42, 45, 62-65
Y	WO 96/35704 A1 (THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE) 14 November 1996 (14.11.96), see entire reference, especially pages 24, 32.	10, 21-22, 32-38, 41, 62-65

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

01 MARCH 2000

Date of mailing of the international search report

21 MAR 2000

Name and mailing address of the ISA/US  
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## INTERNATIONAL SEARCH REPORT

International application No. —  
PCT/US99/25251

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MERLO et al. 'DNA methylation and inactivation of tumor suppressor genes.' In: Hereditary Cancer, Second International Research Conference on Familial Cancer. Edited by Hj. Muller et al. Basel: Karger, 1996, pages 152-160, see entire reference, especially pages 153-157.	10, 21-22
Y	WEST et al. Hypomethylation of the amyloid precursor protein gene in the brain of an Alzheimer's disease patient. J. Mol. Neuroscience. 1995, Vol. 6, No. 2, pages 141-146, especially page 143.	26
A	AHUJA et al. Association between CpG island methylation and microsatellite instability in colorectal cancer. Proceedings of the American Association for Cancer Research. March 1997, Vol. 38, pages 360-361.	1-4, 7-27, 29-38, 41-42, 45, 62-65
A	US 5,786,146 A (HERMAN ET AL) 28 July 1998 (28/7/98), see entire reference.	1-4, 7-27, 29-38, 41-42, 45, 62-65
A, P	US 5,912,147 A (STOLER ET AL) 15 June 1999 (15/6/99), see entire reference.	1-4, 7-27, 29-38, 41-42, 45, 62-65.
A	US 5,501,964 A (WIGLER ET AL) 26 March 1996 (26/3/96), see entire reference.	1-4, 7-27, 29-38, 41
A	LISITSYN et al. Cloning the differences between two complex genomes. Science. 12 February 1993, Vol. 259, pages 946-951, see entire reference.	1-4, 7-27, 29-38, 41

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/25251**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 5-6, 28, 39-40, 43-44, 46-61  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
A sequence search could not be conducted due to errors in the computer-readable form of the sequence listing.
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

**A. CLASSIFICATION OF SUBJECT MATTER:**

IPC (6):

C12Q 1/44, 1/48, 1/68; C12P 19/34; C12N 9/22, 15/00; C07H 21/02, 21/04

**A. CLASSIFICATION OF SUBJECT MATTER:**

US CL :

435/6, 15, 19, 91.2, 91.52, 199, 320.1, 810; 536/23.1, 24.31, 24.33

**B. FIELDS SEARCHED**

Electronic data bases consulted (Name of data base and where practicable terms used):

USPT, EPAB, JPAB, DWPI, MEDLINE, CANCERLIT, CAPLUS, LIFESCI, SCISEARCH, EMBASE, BIOSIS  
search terms: CpG islands, methylation, restriction, ligation, adaptor, adapter, isoschizomer, SmaI, XmaI, PCR,  
hybridization, cancer, carcinoma, tumor, malignancy, atherosclerosis, diabetes, dementia, representation difference  
analysis, p16, Rb, VHL, hMLH1, BRCA1, chemotherapy, pharmaceutical

10/603, 138

6/23/03

Catherine

Lofton - Day et al.

Sequence Listing

# Part 1

## Sequence listing

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 Andrew Sledziewski  
 Jorn Lewin  
 Fabian Model  
 Tamas Rujan

<120> Methods and nucleic acids for analyses of colorectal cell  
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&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 8

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&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 9

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&lt;213&gt; Homo Sapiens

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&lt;213&gt; Homo Sapiens

&lt;400&gt; 15

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&lt;213&gt; Homo Sapiens

&lt;400&gt; 16

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&lt;213&gt; Homo Sapiens

&lt;400&gt; 17

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&lt;213&gt; Homo Sapiens

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&lt;213&gt; Homo Sapiens

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&lt;213&gt; Homo Sapiens

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&lt;213&gt; Homo Sapiens

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&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 25

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&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 26

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&lt;211&gt; 2470

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&lt;213&gt; Homo Sapiens

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&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 33

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&lt;210&gt; 34

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 34

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&lt;211&gt; 3000

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 35

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&lt;210&gt; 36

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 36

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&lt;211&gt; 286

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 39

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&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 40

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&lt;211&gt; 2448

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 41

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&lt;211&gt; 2344

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 42

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&lt;213&gt; Homo Sapiens

&lt;400&gt; 47

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&lt;210&gt; 48

&lt;211&gt; 2382

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 48

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&lt;210&gt; 49

&lt;211&gt; 2192

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 49

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&lt;211&gt; 2192

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 50

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&lt;210&gt; 51

&lt;211&gt; 2244

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (2126, 2128, 2131, 2132)

&lt;223&gt; unknown base

&lt;400&gt; 51

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&lt;210&gt; 52

&lt;211&gt; 2420

&lt;212&gt; DNA

&lt;213&gt; Homo Sapiens

&lt;400&gt; 52

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&lt;210&gt; 306

&lt;211&gt; 2477

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 306

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## 47675-47.txt

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gttttttttag	ttttaat					2477

&lt;210&gt; 307

&lt;211&gt; 2477

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 307

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gagagttaag	gtgaagtatt	tttttttttg	tttaattagt	tttatttagt	ataatttgta	240
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&lt;210&gt; 308

&lt;211&gt; 3685

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 308

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tatattttat	cgtttcgttt	ttttt				3685

&lt;210&gt; 309

&lt;211&gt; 3685

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 309

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&lt;210&gt; 310

&lt;211&gt; 2407

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 310

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## 47675-47.txt

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aagattt						2407

&lt;210&gt; 311

&lt;211&gt; 2407

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 311

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gatttta						2407

&lt;210&gt; 312

&lt;211&gt; 2229

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 312

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&lt;210&gt; 313

&lt;211&gt; 2229

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 313

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&lt;210&gt; 314

&lt;211&gt; 6887

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 314

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&lt;210&gt; 315

&lt;211&gt; 6887

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 315

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## 47675-47.txt

tggtagtg	gtgcgcg	aagttgg	tgagatt	gtcgtgg	ggcggat	5160
agttggg	atgttg	gcgttg	ggtcg	ttcgtt	agcggcg	5220
cggtagc	ggatagg	tggttat	cgggtat	tttgtag	ttgtaga	5280
tgttatg	cgggagt	cgttcgc	gtatgag	gaagttg	ttgacgt	5340
cggatagg	ttggtgg	tggtggt	gggtgg	atgggtg	gggtgag	5400
ggtggaat	gttagata	gtggagat	gtggtag	ttggagc	gttagcg	5460
tgtagg	gtttatg	atgttag	gagacga	gtaggata	tttatgg	5520
ggtgtag	gatggag	tcgggtc	agtcg	gtttagg	gaggtat	5580
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aagattt	tatgtgt	tatagata	tattcgt	taataag	tttatcg	6720
gtttttt	gggagtt	agtttag	tttaaat	aggcgta	gtatttt	6780
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ttttcgt	tagcga	cggtcgg	gcggttt	ggcggc		6887

&lt;210&gt; 316

&lt;211&gt; 3952

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 316

ggtcgtag	ggagagga	aggaatcg	gtggggac	tttggttt	gggtttat	60
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agaggttt	agggata	tagggaga	cgtagcgg	tcggggaa	cgtacgtt	180
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agcgtagc	agttttta	gttcgtaa	cggatcga	tggaagtc	gcgttg	420
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ttcgaagg	tttttttt	agtatttc	tttttgga	gtcgtggg	agaatta	900
cgagaga	aggggga	ggacggg	tagagtag	aggggcg	tgggttgg	960
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gaagtgg	gggtttcg	tggtcgtt	gggatagt	gacgaga	gagagtag	1260
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## 47675-47.txt

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ttttggagta	attagtttga	atgttagtta	ttttttttta	tttggttaaa	tttttttttt	3900
ttgtttgggg	tttttttaag	atgagatggt	tttttgataa	ttaatattta	tt	3952

&lt;210&gt; 317

&lt;211&gt; 3952

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 317

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aaaaggatta	atttataggg	atgatagata	aatttttttg	aaatttgat	gtgttatatt	180
tagttatttt	atatttgata	gaatgatttt	attttatttt	taagaacgtt	tgtaattttt	240
ttgttttggg	tattttaagg	tttataagga	attaagggtg	aaggattttt	agatgaattg	300
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aagtatttga	aattatacga	atgttttagt	ataattagtt	ttggttattt	taatagtttt	420
agtaaagagt	ttttaagtga	atggggtcgt	ggttatcggt	atgggatggg	agagattttt	480

## 47675-47.txt

aaatatttgg	aggggtttttt	gagggtcgatg	taaattttagg	ggagggtattg	gtatatattt	540
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gatttagaag	gtagggggcg	ggagggagtt	tggatatatg	aggaaagaga	atatttttagg	780
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&lt;210&gt; 318

&lt;211&gt; 2820

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 318

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&lt;210&gt; 319

&lt;211&gt; 2820

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 319

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cggaaggcgg gtttcgttat gtcgtttgtt tttttcgttg gagagaatga aagaaacgcg      60
tagagttaga gatttttgtc gagtttagatt ttttttcgtc gtttttaggtt atcggttatt      120
cggtaaagat tcgagtaagg aacgtagggg tattgttttg gtttaataaat ggagttcgtt      180
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tagaggagtt tttttttttt ttttagatta tttgtttcga ttaatttgat tttttaaata      300
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tttggtaggt ttcgttttgt tgaagttttt taattaaata ggggttagagg atgggagttg      2700
ttgtattttt agttggtata gtatttcggt ttgatagttt gtagtatatt gggtagaatt      2760
gtggtgtaat ttttttggta ttttaaatat cgttaatatg tttgggtatt gtttttttagg      2820

```

&lt;210&gt; 320

&lt;211&gt; 2265

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 320

```

tatttttatt tagcgtgttt aggggtattcg tatcgtgtta gtttgtggcg gtttttaaat      60
taattttgat tgagtttttg aggtagtttt ttatttttat tcgtattaat ttagtacgtt      120
tattaaaatt ttttaagtaat tttttttttg ttacgtaaac gttaggagtt gtttgatttt      180
tttttttttt tatttttatt ttttttttag tttagtcggt tttttggtcg ggtaagttgg      240

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## 47675-47.txt

gaatttagcg	attgaagggt	tttggaaaggt	gtcggaggggg	agagacgtta	gttcgaattt	300
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tatttagttg	atatacgaga	tttaaatttt	gttttttagg	aggtg		2265

&lt;210&gt; 321

&lt;211&gt; 2265

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 321

tatttttttga	agagtaaaagt	ttagattttcg	tgtatttaatt	gaatgatttt	tgaatttttt	60
tttttttttgt	atacgagtta	agtaggatgt	ttttgtagta	ggaaataaaag	gggagagggt	120
atagaaaattc	gggttttgga	aatggagaga	ttgggttata	tttatggagt	taggaggggg	180
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cgagattttt	ttattttttt	ttggaggat	ggtaggttat	atatggattt	atttattttt	300
gcgggagttt	ttggttattg	aatatagtta	aagagttttt	attttaatgt	tttgaaaata	360
gatgaaaata	ttaaatagaa	gtggagtgtg	ttggtttttt	ttgtaagtta	gtaacgagga	420
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tttttttaag	agtaaatatt	taaaatatgt	aagtataaat	ttgtttttta	atattttatt	660
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ggtgtagttt	acgggggcgg	ggtagtggga	agtaaggaaa	gatggtcggt	tattttattt	840
ttaaaatatg	tgtatttttg	aggtttttga	ttggaatata	ttgttttttag	atattataga	900
gtttttgtgt	gtgggtttttg	cgatgttttt	ggaatttatt	aaaaatagat	agattaagat	960
ttttttttat	tttataataa	gtaaaaatat	atataagcgc	gcgggcgcgt	atatataata	1020
atataatat	tttttttagta	tttttttatt	tttttttaag	taatgttttg	ttgtgttaaa	1080

## 47675-47.txt

attagttgga	cgagttaggg	tgttattggt	gttttcgagg	ttttgggttaa	tagaattaga	1140
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gttagtagtt	agtcggtcgg	agatagagat	tttacgattt	ttagtttttt	tttcgtcgcg	1260
gtcgtcggtt	tttttttttt	tttttttttt	tttttttttt	ttttttcggt	tttatagtta	1320
tgtttggtta	gattagagta	gttttatagt	taattagggt	agttgtcgtc	gttataatag	1380
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taagttagta	cggtagcggt	gttttgagta	cgtaggggtg	gggtg		2265

&lt;210&gt; 322

&lt;211&gt; 5907

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 322

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cgagtttttt	tttttttcgc	gtttgggttc	gtttgcgttt	ttttcggcgt	gggtattcgt	120
agattcgtta	ggaggttgac	gagagggtgt	tttatagagt	tttggttttt	tttagatttt	180
taaacgaaaa	gaaagagaaa	agttaatttt	tcgtttttat	tttgtacgta	tttggagagc	240
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## 47675-47.txt

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&lt;210&gt; 323

&lt;211&gt; 5907

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 323

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&lt;210&gt; 324

&lt;211&gt; 3049

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 324

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tattagtgtt	agagatttga	taaaattagt	ttttttttta	ttaaatttaa		3049

&lt;210&gt; 325

&lt;211&gt; 3049

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 325

ttggatttgt	agaaaagagg	ttggttttgt	taggtttttg	atattaatat	aacgttttagt	60
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cgcggtaatt	gttgagtaga	tataaatatt	ttaaaataaa	aatgatattt	atttgtgagg	300
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&lt;210&gt; 326

&lt;211&gt; 4721

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 326

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&lt;210&gt; 327

&lt;211&gt; 4721

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 327

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&lt;210&gt; 328

&lt;211&gt; 2146

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 328

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ttgtcgcggc	gattggcgat	aagttattag	ttatttacga	tggtttaagg	aggcgaagaa	120
gagtaagcga	ttaggatatt	aaaacggtta	tcggaggtag	gttttttagta	tattagtcgg	180
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cgagggttgg	cgagcgggat	agtgggaaga	gagaaaagggt	ttaaagggat	ttaagatttg	360
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## 47675-47.txt

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aatattttgt	aggggggagga	gggttgagaa	aagggtgata	aaagga		2146

&lt;210&gt; 329

&lt;211&gt; 2146

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 329

tttttttggt	attttttttt	taattttttt	ttttttgtaa	gatgttttaga	gtttttaaaa	60
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## 47675-47.txt

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&lt;210&gt; 330

&lt;211&gt; 2427

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 330

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tttagtgtaa	ttgtagtagt	ggcgggt				2427

&lt;210&gt; 331

&lt;211&gt; 2427

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 331

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&lt;210&gt; 332

&lt;211&gt; 3015

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 332

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tttttttttt tttttggtag aattgttcga tggttttgat tatgtttagg tttatttcga      60
tgtagtagta ggtatggcgt gttcgggggt cgggatagtt cgttacgtag tagtaatagt      120
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## 47675-47.txt

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&lt;210&gt; 333

&lt;211&gt; 3015

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 333

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## 47675-47.txt

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&lt;210&gt; 334

&lt;211&gt; 3093

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 334

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## 47675-47.txt

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&lt;210&gt; 335

&lt;211&gt; 3093

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 335

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## 47675-47.txt

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gttaggggtt	atttcgttgt	cggatgtagt	gat			3093

&lt;210&gt; 336

&lt;211&gt; 2436

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 336

agttttggat	gataaggatt	aattgttttag	ggcgttggtc	gagaggaata	ggaagagcgt	60
ttcgtggagt	agggcgggcg	agaagagtcg	ggcggtcgcg	gggttttcgc	gtatgttggg	120
taggaagagg	aagagttgta	gtacgaaggt	tattagggag	atgtataaga	agtaggcggg	180
ggttattacg	ttgagtagtc	gtaggggttag	tattttcgtat	tatatttttg	cgggggttcgg	240
ggtgagaaga	ggattgattg	attacggcgt	ttagggggcg	ttttcgttcg	ttttttcgtg	300

## 47675-47.txt

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cgggttttagg	ttttttttgg	gggtgagtgt	tgtttgggt	tttgagcgag	ttcggcggttt	480
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&lt;210&gt; 337

&lt;211&gt; 2436

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 337

cgggcggttga	aattaggttag	ggcgttttag	ttcgtttaag	gggaatcgag	aggggaggag	60
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gggtggcggg	gttggggggt	gttttagttg	tatttttcgt	gttttttggt	ttttattcgt	180
tggcgtttaa	gtgggaagtt	agtagttgtt	cgtttttttt	tttttatatt	ttcgttattt	240
gttttagttt	cgaagcgaag	cgtaggttgc	gagttagtcg	ggtcgagttt	ataattttgt	300
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&lt;210&gt; 338

&lt;211&gt; 17219

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 338

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&lt;211&gt; 17219

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

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&lt;210&gt; 340

&lt;211&gt; 2264

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 340

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&lt;210&gt; 341

&lt;211&gt; 2264

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 341

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<211> 3104

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 342

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&lt;210&gt; 343

&lt;211&gt; 3104

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 343

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cgttaggatt	attttagtat	tttttcgtgt	tcgatagcgt	tcgt		3104

&lt;210&gt; 344

&lt;211&gt; 2493

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 344

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&lt;210&gt; 345

&lt;211&gt; 2493

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 345

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&lt;210&gt; 346

&lt;211&gt; 2315

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 346

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aatatTTatt	ttaaagtaga	gattTgtaga	ttatagTTac	gggtTaaatt	tggtTTtcgg	1920
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acgtTTaaaa	ggTTtatTTt	ttggTTTt	atataaaaaa	tttattTTt	ttTgtTTtaa	2040
agatTTgaag	ggacggTgtt	taggggtagg	aatgagTaat	atcggTTTTt	atcggTTata	2100
ggtagaaatg	ttataatTTa	tagggTatta	attggaatgt	atataaagga	ttcgtTTtat	2160
taacgtggag	taagtagTTt	aaagtgattg	ttgtTTtagg	tttgattgat	atattataaa	2220
agtaagattt	gaaaggatta	aattgtTTat	aagtatTTta	attgattTTg	agaataaagt	2280
ttaagaataa	gaatTTTTta	aatattaata	aaaaa			2315

&lt;210&gt; 347

&lt;211&gt; 2315

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 347

ttttttgttg	atgttttaaaa	aattttttatt	tttgagtttt	gttttttagaa	ttagttaaga	60
tattttgtgaa	tagtttgatt	tttttaggtt	ttgtttttat	gatgtattag	ttagggtttag	120
agtaataatt	attttgggtt	atttatttta	cgtagtgag	gcgagttttt	tgtgtgtatt	180
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tatagtttgt	taatttttgt	tttaggataa	atgttttgta	gttttttagtt	attttttttta	480
gagaggggga	ttttttttga	ggtttttttcg	gcgtagtttt	tttttttggga	gtttttatacg	540
gcggattcga	gttgttgtgg	tttttttggga	tttttagttt	gattttttttt	atttggggag	600
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gtttaaagt	ttttgggggt	tcgttttgtt	tttgttattt	ttttagggtt	tttaattaga	1140
ttatgagtaa	atagtttttg	gaattgggtt	tttcggtatt	cgtttttgat	ttagcgggtta	1200
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ttttgttttg	taaaaatttt	tttatttcgg	tttttattag	tatacggcgt	acgtttatta	1320
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aaatatattg	ttagtatagc	gttcgtagt	tcgggtatta	ttgttaagggt	agggtgggtat	2040
tatttatgaa	gggtgttatag	agatggaggt	ggattatggt	ttttgttttg	gatttgtagt	2100
gggttgtttt	gttttaaagt	gtgaatatac	gttttttggt	ttgggggggag	cgggggtttt	2160
gtattttttt	ttttttttga	tttgaggtat	ttagggtttt	agggtttttgt	tgttagttgt	2220
ttgatggtgt	cgttttttat	tgaataaatt	attttaggtg	ttatagggtg	gacgttaata	2280
acgttcgtgg	ttaggagatt	ttacgttagg	tatag			2315

&lt;210&gt; 348

&lt;211&gt; 2304

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 348

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ttaatTTTTT	gtgtttttta	gtagaaacgg	ggttttatta	tgttggttac	gttggtttcg	120
aaattttgat	tttaagtgat	ttatttat	tcgtttttta	aagtgttggg	atttttaggag	180
tgatttcgta	tgttttagtta	ttaaataata	tttattaagt	gtttattttg	tttttaagag	240
tgttgggaat	ttagtagtga	ataaaataaa	tatttttttt	cggggatttt	gaatttaagt	300
ttagttaggg	attattttat	taagagatta	agttaataat	taattttttt	atagattttag	360
ataggatagt	attttaattt	ttcgttgatt	tgttttttta	gatttttagat	tatgggtatt	420
gtagattttt	gtattttttg	tagaggtagg	gttttattat	tattagagat	tttattatta	480
tttttatgtg	aaggggaaata	gtgttttttt	agtagataac	gttaaattag	ttattagacg	540

## 47675-47.txt

tggttatt	tcggtttatt	gtaagtttcg	tttttttaggt	ttacgttatt	tttttttttt	60
agtttttcga	gtagttggga	ttataggcgt	tcgttattac	gtttgttaaa	ttttttgtat	120
tttttagtaga	gacgggggtt	tatcgtgtta	tttaggatgg	tttggatcgt	ttgatttcgt	180
gattcgtttta	ttttggtttt	tttaagtgtt	gggattatag	gcgtgagtta	ttacgtttag	240
ttcgtgggtg	ttgggtttta	atagaagata	cgggtcgggc	gcggtagtta	cgtttgtaat	300
tttagtattt	tgggaggtcg	aggcgggcgg	attacgaggt	taggagatcg	agattatttc	360
ggttaaaacg	gtgaaatttc	gtttttatta	aaaatataaa	aaattagtgt	ggtttggtgg	420
cgggcgtttg	tagtttttagt	tatttgggag	ggtgaggtag	gagaatggcg	tgaatttttg	480
aagcggagtt	tgtagtgagc	ggagatcgcg	ttattgtatt	ttagtttgag	cgatagagta	540
agatttagtt	ttaaaaaa	aaaaaaaaaa	aaaatagaag	atacggatag	aaatttttgt	600
tatatcgagt	ttcgtttttt	ttttatagta	tttttacgtt	agtgaataat	tgacgtatag	660
gtagagaatt	tttattttgt	tgttatgggg	agatttaggt	agagtcggta	tgaaagtaat	720
agttgatatg	aataacgtgt	gttatttttag	ttaaagtcgta	tgttggagtt	tattttatag	780
ataagaaaa	tgaggattag	agaagttaag	tgatttattt	acggttttat	taataatata	840
tcgtaagggt	agaattggaaa	tttatatttt	tttagtgtgt	tagtttaagt	gttttttttt	900
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gaaaaattgt	ttgggtgata	gtgaagtggg	ttgttttaac	gttcgcggag	gatgtttgtt	1020
agaagtaatt	taatttaagc	gagggtataa	tttcgaggga	ggttagtatg	gtttgtttgt	1080
ttttttttga	ttgtttttta	agatatattt	attacgttta	ggttggaaaa	taggatagtt	1140
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cgttcgtggt	ttttttatta	agtgtttttt	tcgggaggcg	cgttattttt	gagtatgtta	1320

&lt;210&gt; 349

&lt;211&gt; 2304

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 349

tggtgttatt	tcggtttatt	gtaagtttcg	tttttttaggt	ttacgttatt	tttttttttt	60
agtttttcga	gtagttggga	ttataggcgt	tcgttattac	gtttgttaaa	ttttttgtat	120
tttttagtaga	gacgggggtt	tatcgtgtta	tttaggatgg	tttggatcgt	ttgatttcgt	180
gattcgtttta	ttttggtttt	tttaagtgtt	gggattatag	gcgtgagtta	ttacgtttag	240
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tttagtattt	tgggaggtcg	aggcgggcgg	attacgaggt	taggagatcg	agattatttc	360
ggttaaaacg	gtgaaatttc	gtttttatta	aaaatataaa	aaattagtgt	ggtttggtgg	420
cgggcgtttg	tagtttttagt	tatttgggag	ggtgaggtag	gagaatggcg	tgaatttttg	480
aagcggagtt	tgtagtgagc	ggagatcgcg	ttattgtatt	ttagtttgag	cgatagagta	540
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tatatcgagt	ttcgtttttt	ttttatagta	tttttacgtt	agtgaataat	tgacgtatag	660
gtagagaatt	tttattttgt	tgttatgggg	agatttaggt	agagtcggta	tgaaagtaat	720
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gaaaaattgt	ttgggtgata	gtgaagtggg	ttgttttaac	gttcgcggag	gatgtttgtt	1020
agaagtaatt	taatttaagc	gagggtataa	tttcgaggga	ggttagtatg	gtttgtttgt	1080
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gtcggagtgt	cgttgcgagg	ttagcgggtg	ttattgtttt	tttacggtgt	ttagagagag	1200
tttgttgtga	acggtagtta	gcgatatatta	ggtttttagt	tttttcgtag	tcgggttttcg	1260
cgttcgtggt	ttttttatta	agtgtttttt	tcgggaggcg	cgttattttt	gagtatgtta	1320

## 47675-47.txt

tatagtagat	agtagtttcg	tgttgatggt	taacgttata	agttttatatt	tttagagttt	1380
ttttattttt	ttaaaaatat	cgtttttttt	taaaatcgta	ttatgtgtaa	aattaaaata	1440
agatggatgg	ggagatgtag	gaatattaga	gatttggtatt	tatagtaaat	tagaagaatt	1500
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tttataaaag	gtgtaaaaat	ttgtagtgtt	tatggtttag	ggtttgaggg	agtagattaa	1920
cgagaaatta	aaatattatt	ttatttgagt	ttgtaggaga	attgagtgtt	gatttgattt	1980
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ttattaaaaa	atataaaaaa	ttagttgggt	gtatatttta	tatttgtaat	tttagttatt	2280
cgggaggttg	aggtaagaga	atcg				2304

&lt;210&gt; 350

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 350

gtggaatata	ttttttatat	agttatatta	tttaaaattt	attgttttta	agtttttatt	60
taaatgtttt	ttcgtgaagt	tattttttgag	ttttttttta	aattgttagt	ttttattttt	120
gtatttttta	ttgtttttta	tttttaattt	ttgttattgt	agttagaatt	atttgatata	180
tagtttttat	ttttttggtt	ttttgttatg	ttttttttta	taagaatttg	aagtttatta	240
ggtcgggagt	tttggtttgt	gtgtttgttg	ttatttttta	gtgtttaaaa	ttgttttgta	300
tatagtaggt	atttaataat	ttttgaatta	gtgaaaatta	gatggtgggt	tggtattttt	360
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aaggggtatt	ataattatgt	gggggtttatt	ttgttttttt	ttgttttttt	tttttaggtt	480
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## 47675-47.txt

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ggattcgttt						2470

&lt;210&gt; 351

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 351

ggacgagttt	attttaagtt	ggcgtgggga	aaaatataag	tacgtagatc	gattttattat	60
ataacgggag	ggggagggtta	ggttacggtt	agattttatta	taaggagacga	agggtttgag	120
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tgtgttttat						2470

&lt;210&gt; 352

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 352

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ggttcggagg                                     2470

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&lt;210&gt; 353

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 353

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ttttcagatt tagggttagg tcgtgtattt ttttaatttt ttgtgcgagt tttttttcgt      60
cgggttttaa gcgtttcgtc gttatgtttt cgttttcgat tttattttta tttcgttatt      120

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## 47675-47.txt

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gttttttgg						2470

&lt;210&gt; 354

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 354

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&lt;210&gt; 355

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 355

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&lt;210&gt; 356

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 356

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&lt;210&gt; 357

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 357

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<210> 358  
 <211> 2470  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 358

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<210> 359  
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 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 359

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&lt;210&gt; 360

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 360

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&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 361

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&lt;210&gt; 362

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 362

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&lt;210&gt; 363

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 363

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 <223> chemically treated genomic DNA (Homo sapiens)

<400> 364

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 <212> DNA  
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 <223> chemically treated genomic DNA (Homo sapiens)

<400> 365

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 <211> 3120  
 <212> DNA  
 <213> Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 366

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&lt;210&gt; 367

&lt;211&gt; 3120

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 367

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&lt;210&gt; 368

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 368

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&lt;210&gt; 369

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 369

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acggtttagc gtaaatttta gttttggttt tcggagttta atgtatttcg tagatagttt 300

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## 47675-47.txt

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&lt;210&gt; 370

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 370

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## 47675-47.txt

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&lt;210&gt; 371

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 371

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&lt;210&gt; 372

&lt;211&gt; 3000

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 372

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## 47675-47.txt

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&lt;210&gt; 373

&lt;211&gt; 3000

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 373

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&lt;210&gt; 374

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 374

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<400> 375

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<210> 376  
 <211> 2501  
 <212> DNA  
 <213> Artificial Sequence

<220>

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 376

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aagtttgttt attaaagaag ttatgattta atttacgaag attaggaggtt gggtgggtga      180
agaaaaaaag gtttagaggaa ggaagtttat attggggaag gttttaagta taaagggtag      240
gaggattata gaggtatatt tacgaaattt ggagaaggtt tttagtaagt aaggagaagt      300
taaataaaag ttacggggag agttggaggt ttgaagatac gtttaaggat ttggttttta      360
tttttttttt attttaagag tagtgggaag ttattaaatg attttaatta gagggttgggt      420
ataattagtt ttgtattttg aaaagttgaa tttagttttc gtttgagaaa ttgagtgaaa      480
gagtttagaa cggtcgtggt tgaggggtgat tcgtgggaga tttttatata agttatggta      540
gtggtatggg ttggtggtag aagagggaat agggagaaga tttggaattt aatttttttt      600
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tagtacgatt ttatcgtagc tttagaagtc gggtagtggt tttttagttc gggttcggcg     2460
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```

&lt;210&gt; 377

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 377

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attattttatt cgatttttga acgtgcgggt ggatcgtgtt ggcgatacgc gtttatagga      120
cgatgtgtag cggttatagg tttttgagta gttttcgatt tatggtagat ttcgttggtc      180
gttatagatc gagtttttag cgtagcggac ggcgtttttt cggattttttg gttgcgtttt      240
cgcgtcgcgt ttttttcgga tttcgcgtcg ggtcggtagc gtagatgtgc gggtttagatg      300
tggcgttcgt tcgttagtta ggaggggggt tggaggtcgg cgaggcgcgg ggaggttttc      360

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## 47675-47.txt

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gattttgtat tatacgtttt tttggtatta tatgtttttt ttttaatagt tgtaatttta 2460
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<210> 378  
 <211> 1508  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 378

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tttatttcga agcgtttggg agatatttta gaaacggatg ggaaatgta aattttgtat 180
gtttgtttat tttttttttt atatcgatta gatgtaaacy agtgttatta aaagtatatt 240
ataggatatt atatatagatt tttttataag ggatttataa agtttagatg tgaaatgtat 300
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## 47675-47.txt

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gaatgggttt	gggcggggag	gtgaagagaa	gttaggaatg	ttttatgttt	ttttaatgga	1140
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aaaaattttt	ttgtattttg	gcgtttggta	gggtttttta	aaaaaagtaa	attttttttt	1500
taaatgta						1508

&lt;210&gt; 379

&lt;211&gt; 1508

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 379

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ggattttt	gatgtttt	ga	aagtgtaa	atatcgcg	tcgtttgg	120
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gttttttt	tattaggaa	at	ataaaaata	tttttggt	tttttat	420
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atatgttgg	tcgtggttg	at	gaaaattg	tgaggagg	tttttggt	660
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aatggaatt	tatgttaata	gg	tttatgtt	tatttgtaa	atatattaa	1500
tgtagaat						1508

&lt;210&gt; 380

&lt;211&gt; 286

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 380

tttgtatt	ggtggaagt	gtcgttag	tttcgtgt	ttttatttt	tggaaaagt	60
gaattagt	gtattgttt	gcgtgatt	tgaggttg	ttttaatag	ttaaagaag	120
aaatgggat	ttatttttc	gggttcgt	tttcgcgag	tgtttatt	gtatttgta	180
tgtaaaacg	gggagcgt	ggaagga	cgttttgt	agttattgg	tttggttatt	240
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<210> 381  
 <211> 286  
 <212> DNA  
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<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 381

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<210> 382  
 <211> 2501  
 <212> DNA  
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<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 382

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gttttt	atataaa	aagatgt	ttttat	ttacgt	atttttt	1020
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## 47675-47.txt

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&lt;210&gt; 383

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 383

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&lt;210&gt; 384

&lt;211&gt; 2448

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 384

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&lt;210&gt; 385

&lt;211&gt; 2448

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 385

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tattatata taggtatttt ataaatattt gttgaatttt tatgatttta ttaattttat      180
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## 47675-47.txt

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&lt;210&gt; 386

&lt;211&gt; 2344

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 386

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&lt;210&gt; 387

&lt;211&gt; 2344

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 387

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<210> 388

<211> 2350

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 388

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&lt;210&gt; 389

&lt;211&gt; 2350

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 389

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&lt;210&gt; 390

&lt;211&gt; 2206

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 390

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&lt;210&gt; 391

&lt;211&gt; 2206

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 391

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&lt;210&gt; 392

&lt;211&gt; 2233

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 392

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17075-17100							
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<210> 393

<211> 2233

<212> DNA

<213> Artificial Sequence

 $\langle 220 \rangle$ 

<223> chemically treated genomic DNA (Homo sapiens)

<400> 393

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<210> 394

<211> 2398

<212> DNA

<213> Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 394

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&lt;210&gt; 395

&lt;211&gt; 2398

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 395

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&lt;210&gt; 396

&lt;211&gt; 2114

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 396

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&lt;210&gt; 397

&lt;211&gt; 2114

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 397

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 <211> 2382  
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<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 398

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 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 399

## 47675-47.txt

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&lt;210&gt; 400

&lt;211&gt; 2192

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 400

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&lt;210&gt; 401

&lt;211&gt; 2192

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 401

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&lt;210&gt; 402

&lt;211&gt; 2192

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 402

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tagtttttta	tttgttgatt	taggattttt	at			2192

&lt;210&gt; 403

&lt;211&gt; 2192

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 403

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tttaatttaa gttttttaat ttttaagaat tgataattta attttgtatt ggtacgattt    300
aagaataagt aagaattatt tttaaaatta aagtaatttt aaagtttttg ttttatttaa    360
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taggggtttt ttttttttta aaaattatat gg                                2192

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&lt;210&gt; 404

&lt;211&gt; 2244

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (2126, 2128, 2131, 2132)

&lt;223&gt; unknown base

&lt;400&gt; 404

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ttttattttt tttttttttt tcgttttttt tttatttttt tttttttttt tttttttttt    60
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## 47675-47.txt

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&lt;210&gt; 405

&lt;211&gt; 2244

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (113, 114, 117, 119)

&lt;223&gt; unknown base

&lt;400&gt; 405

acgaaggtag	ttttagcgat	tttttgcgga	aaatttagta	gcgtgggtag	tggtgtttcg	60
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## 47675-47.txt

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gcggaagaga	aagagaaaat	ggaa				2244

&lt;210&gt; 406

&lt;211&gt; 2420

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 406

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## 47675-47.txt

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gtgtttaaga	gattagtcgg					2420

&lt;210&gt; 407

&lt;211&gt; 2420

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 407

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<220>  
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&lt;400&gt; 409

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&lt;210&gt; 410

&lt;211&gt; 2366

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 410

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&lt;210&gt; 411

&lt;211&gt; 2366

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 411

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&lt;210&gt; 412

&lt;211&gt; 2264

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 412

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tatttgcgtt	ttcgttagtt	ttttttggga	ttttatcggt	gtcggtagtt	ttcgggtttt	780
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ggagtttttta	aaatttttgg	ggaaggagcg	tatttcggga	ggcgcgata	ggtgggtttg	1980
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## 47675-47.txt

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cgttcgtggg	gaaaaggtag	ggaggggggtg	gtgttttttag	tcggtttggg	gggtgcgttg	2220
ttcggagacg	gaaagtttgg	gagttcagag	aggttcgggt	gtag		2264

&lt;210&gt; 413

&lt;211&gt; 2264

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 413

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tcggttgggg	atattatttt	ttttttgttt	tttttttacg	agcgcgttat	tttcgggatt	120
cggcgttacg	cgttatcgcg	ttttcgcggt	tcgtttttta	gtttattttag	ttgggcgggg	180
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gtaattcgtt	taggtagcgt	agaaataaag	agataaatgt	aacgcgtatt	tatattttta	420
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attttttagta	gagacggggg	tttattatgt	tggtttaggtt	ggtt		2264

&lt;210&gt; 414

&lt;211&gt; 1334

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 414

gggcggttttg	atTTTTTTTT	tattttgttt	tttcgggttt	tattcggtttg	tttttggatt	60
ttcggtttttt	tttggtttttc	gggttttttag	agttttttttt	ttatggtagt	agttttttcgc	120
gttttcggcg	tagtttttta	gcggacgatt	ttttcgtttc	ggggttgagt	ttagtttttg	180
gatgttggtg	aaatTTTcga	gattatgcgc	gggtttggtt	gttggtttttt	cgtcgggtgt	240
tattgttatc	gtcgtcgttt	ttgttgctgt	cgttcgcggg	atgttttagta	gttcgttggt	300
cggttttcgc	gattttgtgt	ttttcggaa	tcgtttgttg	ttgtagagt	gtacgaatta	360
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tgttgggttg	tgttggtgtt	cgttatgtta	tttatcgtag	ttcgttcggg	gaagtTCgtt	480
gtttttttta	tttttttaag	tgattgttaa	acgttttatcg	gttggaattg	ttttggtaag	540
tttagaattt	tcgttttcga	ttttttaatt	tcgtagaaga	atacgcgtat	ttagtataga	600
ttagtttatt	ttagcgcgtt	tttttagttt	tttatTTTT	attgttttag	atTTTTaata	660
ttattttatt	ttatttagag	aaataagggg	aattgttgta	ggttcggggg	tgaggggtgg	720
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gtgggtatga	ttgaggttgg	ttaggtttta	tgtaggcgag	tcgagggcgg	aattcgattt	1320
tagtgggcgt	tgat					1334

&lt;210&gt; 415

&lt;211&gt; 1334

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 415

gttagcgttt	attgaagtcg	ggtttcgttt	tcggttcgtt	tatatggagt	ttgattagtt	60
ttagttatgt	ttatttcggg	ttgggagatt	cgtaaagtgt	tttttttttt	aattttttttg	120
tattattttt	aagtttaggg	aagtaaagag	aggggtatat	ttggattgta	aaattaatgt	180
tttttgtcgt	ttaggagaga	agggaaatgag	agagagagag	agatagagag	agagagagag	240
agagagagag	agagaaattt	tattgaaatt	tagttttttt	agaatttgtg	tgattttggtt	300
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agttattatt	tgatttattg	ttttttcgtt	tatttttttt	tataaagtta	tttttttttt	420
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gattggtgtt	tttttaaaat	agtgaattta	gaaaattatt	tcgtttaata	ttttttaaaa	540
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gaggtaggga	aagttagcga	ttttatcggg	cgggttacga	tgagttagtat	gacgggtagt	900
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tatagtatta	tgattagttc	gtgtaatttt	gtagtagtaa	acggtttttcg	aggaatatag	1020
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cggcggtggt	agtgggtatt	ggcggggaag	tagtagttaa	attcgcgtat	gatttcgaga	1140
gttttagtaa	tatttaggga	ttgggttttag	tttcggagcg	agagggtcgt	tcgttgagaa	1200
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taggagagga	cgggagttaa	ggggtagacg	agtggagttc	gaggaggtag	ggtggaggga	1320
gagttaaggc	gttt					1334

&lt;210&gt; 416

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 416

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tgcggatatg	tgatgttttt	tttggaatatt	cggtttttaa	atgttttttt	tttgtatttt	120
ttttttttat	tttttaggtt	agtcgatatt	tagggaaaa	aggaaaggat	ttacgtgaaa	180
tattgggggt	tgaatttttt	tcgataataa	gtttttttaa	aaagattttt	aaatgaaatt	240
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ttatagtgtt	tgttgttttt	ttgtttgtgt	tataagtttt	tattatttag	ttttatttta	480
taagtggagaa	tatttagtat	ttggattttt	gtttttgtat	tagtttggtta	aggataatag	540
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&lt;210&gt; 417

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 417

cgggagaggc	ggggaatagg	aaggagggttc	ggggtaaaag	ttatacgacg	gagggataag	60
ggggttcggg	ttttttcggg	tgggagaggg	gttgtgggtt	gtagtttttag	tttttgtttt	120
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## 47675-47.txt

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gttttcgaggt gggcgaggag tttgtcggga gttcgggttt gatgttgccg gttgggtttta 420
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&lt;210&gt; 418

&lt;211&gt; 2327

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 418

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&lt;210&gt; 419

&lt;211&gt; 2327

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 419

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## 47675-47.txt

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&lt;210&gt; 420

&lt;211&gt; 2280

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 420

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&lt;210&gt; 421

<211> 2280  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 421

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 <211> 2477  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 422

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gttttttttag	ttttaat					2477

&lt;210&gt; 423

&lt;211&gt; 2477

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 423

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## 47675-47.txt

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gggttatggt ttgtgta 2477

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&lt;210&gt; 424

&lt;211&gt; 3685

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 424

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## 47675-47.txt

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&lt;210&gt; 425

&lt;211&gt; 3685

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 425

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## 47675-47.txt

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&lt;210&gt; 426

&lt;211&gt; 2407

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 426

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## 47675-47.txt

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aagatttt						2407

&lt;210&gt; 427

&lt;211&gt; 2407

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 427

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tttatgtatg	gtattaagat	tttatgaaga	ttagaaaaat	aatgtttata	aaaggttttt	120
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## 47675-47.txt

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gatttta						2407

&lt;210&gt; 428

&lt;211&gt; 2229

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 428

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agggagaag						2229

&lt;210&gt; 429

&lt;211&gt; 2229

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 429

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gaggaaaga						2229

&lt;210&gt; 430

&lt;211&gt; 6887

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 430

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<210> 431  
<211> 6887

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 431

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&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 432

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gttttggggg gtagagtgtt ggggtgtatt aggtggggat tttttttggt tgtgtgtagt      1560
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## 47675-47.txt

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ttttggagta	attagtttga	atgttagtta	ttttttttta	tttggttaaa	tttttttttt	3900
ttgtttgggg	tttttttaag	atgagatggt	tttttgataa	ttaatattta	tt	3952

&lt;210&gt; 433

&lt;211&gt; 3952

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 433

aataaatggt	agttattaaa	gaagtatttt	attttaagga	gatttttaggt	aggaggggag	60
agtttagtta	agtagggagg	ggtgattaat	attaggtta	attgttttaa	aattagattt	120
aaaaggatta	atttataggg	atgatagata	aatttttttg	aaatttgat	gtgttatatt	180
tagttatttt	atatttgata	gaatgatttt	attttat	taagaatgtt	tgtaattttt	240
ttgttttggg	tattttaagg	tttataagga	attaagggtg	aaggattttt	agatgaattg	300
atttttaaga	atttttat	tttttaatta	gagaaaagag	agaaatagt	aaatagttga	360
aagtatttga	aattatatga	atgttttagt	ataattagt	ttgggtattt	taatagtttt	420
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## 47675-47.txt

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ttggggatta	gggtgttttt	attgtgggtt	ttgttttttt	ttggttgtgg	tt	3952

&lt;210&gt; 434

&lt;211&gt; 2820

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 434

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## 47675-47.txt

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&lt;210&gt; 435

&lt;211&gt; 2820

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 435

tggaagggtg	gttttgttat	gttgtttgtt	ttttttgttg	gagagaatga	aagaaatgtg	60
tagagttaga	gattttttgt	gagtttagatt	tttttttgtt	gttttaggtt	attggttatt	120
tggtaaaagt	ttgagtaagg	aatgtagggg	tattgttttg	gttaataaat	ggagtttgtt	180
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## 47675-47.txt

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&lt;210&gt; 436

&lt;211&gt; 2265

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 436

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2265

<210> 437

<211> 2265

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 437

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<210> 438

<211> 5907

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

<400> 438

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&lt;210&gt; 439

&lt;211&gt; 5907

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 439

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&lt;210&gt; 440

&lt;211&gt; 3049

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 440

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&lt;210&gt; 441

&lt;211&gt; 3049

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 441

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&lt;210&gt; 442

&lt;211&gt; 4721

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 442

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&lt;210&gt; 443

&lt;211&gt; 4721

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 443

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&lt;210&gt; 444

&lt;211&gt; 2146

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 444

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aatattttgt	aggggggagg	gggttgagaa	aagggtgata	aaagga		2146

&lt;210&gt; 445

&lt;211&gt; 2146

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 445

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&lt;210&gt; 446

&lt;211&gt; 2427

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 446

ttttttgggg	tttgggtggg	gagttttttt	tagtaataaa	tatgagaggg	atagagaatg	60
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aaaatttttt	atttttttta	aatttttggg	ttagggttat	ttgatttttt	gtaagttgtt	780
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## 47675-47.txt

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&lt;210&gt; 447

&lt;211&gt; 2427

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 447

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&lt;210&gt; 448

&lt;211&gt; 3015

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 448

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## 47675-47.txt

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&lt;210&gt; 449

&lt;211&gt; 3015

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 449

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&lt;210&gt; 450

&lt;211&gt; 3093

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 450

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## 47675-47.txt

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&lt;210&gt; 451

&lt;211&gt; 3093

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 451

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&lt;210&gt; 452

&lt;211&gt; 2436

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 452

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&lt;210&gt; 453

&lt;211&gt; 2436

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 453

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&lt;210&gt; 454

&lt;211&gt; 17219

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 454

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&lt;210&gt; 455

&lt;211&gt; 17219

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 455

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tttttttaat	tttttaagg	ttgtggtggg	gtagtgtaat	agggaaaggg	gggtaaagag	15900
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## 47675-47.txt

ttagggtttt	tttttttttt	atgtgtgtat	ggttatatatt	tgggtgattg	ggtttgattt	16020
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gagttgtgaa	tatatataat	gaatattttt	attttaaaaa	ttgagtttgg	aagatagatg	16140
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agaagagtgg	gttatataat	tatatattata	tatttatatta	tagggaaata	atgtgttttt	17160
ggtttgaagg	aaattgaggg	ttatgttagt	agtttaagg	atggatgggtg	tgtagtga	17219

&lt;210&gt; 456

&lt;211&gt; 2264

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 456

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tatgggtggg	tgtgtttgta	attttagtta	tttgggaggt	tgaagtagga	gaattgtgtg	120
aatttaggag	gtgtagggtg	tagtgggtta	gagattgtgt	tattgttttt	tagtttgggt	180
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ttttaatat	taagaggaat	tatatgtgg	tgaaggggg	gttatgaatt	ttgggtttta	360
gtttttgtat	tatgttttag	tgtgatattg	gatttttaatt	tttttattat	taaagttaaat	420
gggttattat	aattttttta	gaagtagttt	taatttttaa	agataagatt	aaaaatatta	480
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tattttgtgt	tttgttagtt	ttttttggga	ttttattggg	gttgggtagtt	tttgggtttt	780
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## 47675-47.txt

tggtatatatt	atTTTTTTgt	TTTTatgttg	tttggatgaa	ttattgtatt	TTTTgtttta	1920
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TTTgtttttt	tttatttTga	taatgggggt	ttataagggtg	ttgtttttat	gttttgagag	2040
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tttggagatg	gaaagtttgg	gagtttgagt	aggtttggtt	gtag		2264

&lt;210&gt; 457

&lt;211&gt; 2264

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 457

ttgtagtTga	gtttgttttg	gtttttaaat	TTTTgtttt	tgggtaatgt	atTTTTtaaa	60
ttgggtgggg	atattatttt	TTTTTgttt	TTTTTTatg	agtgtgttat	TTTTgggatt	120
tggtgtttatg	tgttattgtg	TTTTgtgtt	ttgtttttta	gtttatttag	ttgggtgggg	180
TTTTgtggat	tagttaggt	ggattttgtt	gtgttgagat	atgttttttg	gggtatgggg	240
atagtgtttt	gtgggttttt	attatgtagg	tggaaaggga	taaatagggt	tatttTgatg	300
tgTTTTttga	agtgtgtttt	TTTTtaaaag	gttttgggag	TTTTgggat	aggaaaatag	360
gtaatttTgt	taggtagtgt	agaaataaaag	agataaatgt	aatgtgtatt	tatatTTTTa	420
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tgaaaaaaat	tatttaattg	ggtagaaatt	atgtgtgttt	tgTTTTttgg	tattagtatt	780
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atTTTTtagta	gagatggggT	TTtattatgt	Tggttaggtt	ggtt		2264

&lt;210&gt; 458

&lt;211&gt; 3104

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 458

gtgggtgttg	ttgagtatgg	ggaggtgttg	aaatagtttt	ggtgtgttga	tttaagtttt	60
gattggtaga	gttattttgg	gattgatagg	gggtttttat	ggtgtttgtg	ttgttaattt	120
gtttatttta	atagtggagt	tagttttgtt	gttggtgtgt	ttgagttgag	ttgagtttga	180
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&lt;210&gt; 459

&lt;211&gt; 3104

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 459

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gtagtttggg	gtgtgatttt	ttgggtgaatt	tgtgggtttt	taggatatga	tagagtttgt	2520
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tgttaggatt	attttagtat	ttttttgtgt	ttgatagtgt	ttgt		3104

&lt;210&gt; 460

&lt;211&gt; 2493

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 460

attggtatga	ttagagtaga	aggaggggat	tttaattttt	agttttattt	attgttttgt	60
aagaattagg	aaaatgaatt	tttggttggt	ataaaaattag	ttgtaggtga	tgtaatttat	120
tttattttgt	tttttatatt	tatagttata	taggaataat	tttttgaaat	ttagtttttt	180
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agatgtattg	gttttttaggg	gtaagtatta	gtagataagg	aatagggtag	tttttttttt	300
atattttata	gagaagaaat	gttttttaaat	tttttttagga	ggtagagttt	agtttttttt	360
ttaaatagtt	ttggagttgt	ttgggttttt	tattttttta	atgaagtgtt	gggtagaaga	420
taaatgtaga	gtttttttgt	atttgataag	gagagagaag	tagattttgt	gtgagttttt	480
tgttgttgat	gttgatttat	aggggggtga	ggatgggatt	gaggtaggtt	tatttgtttg	540
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tttttttttt	ttttggagag	gataggtagg	tgggtatggt	tataggagaa	ttttttgaag	720
gtgttaattg	gtttagagaa	agaaaggtgg	tttggtaggg	agttgtttat	tagagattga	780
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tttgaggtat	atgatagggt	ttatagaaat	gtttgttttg	ttttttgatt	agtatttaaat	2340
agtttgggag	tgtttttagt	gtgggttagg	gtgggtatgg	tttgataatt	attattagaa	2400
tatttttttt	ttttttataa	aatgttttga	ggagaattta	atggttgttg	aaaattaagg	2460
taagttttat	tttttaaaata	tttaggagtt	aat			2493

&lt;210&gt; 461

&lt;211&gt; 2493

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 461

attgattttt	gaatgtttta	gaaatgaagt	ttgttttaat	ttttgatagt	tattaaattt	60
tttttaagggt	attttgtgaa	aaaaggaggg	gtgttttggt	ggtggttatt	aaattataat	120
tatttttagt	tatatattgg	gtatttttag	attgttggat	gttagttagg	agatgaaatg	180
aatgtttttg	tagtatttat	tatgtgtttt	agaatttttt	agttttttgt	ttgatttttt	240
gtaataattt	tgtgaggtag	gtggaatagt	tgttatttta	aagtgaggaa	attgaagttt	300
aaagttttta	tttttaaaag	gaggagttga	agaagagtgt	tagttttatt	ttattttttt	360

## 47675-47.txt

gtgtttttttt	agaaggaaga	ggttatttttg	gtagttttttt	attttttagaa	ttttgtttttt	420
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aggggtattgt	attattattt	tattttatgg	gtgaggagat	tgagtttttag	agagttgttt	2340
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tatgggtta	aggaatttat	ttttttgggt	tttgtaaagt	ggtaggataag	gttgggaatt	2460
aggatgtttt	ttttttattt	tagttatgtt	agt			2493

&lt;210&gt; 462

&lt;211&gt; 2315

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 462

ttgtattttg	tgtgaggttt	tttgggttatg	gatgttggtg	gtgttatatt	tgtgggtattt	60
gaggtgaatt	gttttagatgg	aaatgggtatt	attaggtaat	tggtaataga	agtttaagt	120
tttgggtgtt	ttaggttaga	ggaagggaagg	aatgtaagg	ttttgttttt	tttaagtta	180
ggagtgtgtg	tttatagttt	tagataggat	aatttattgt	aaatttaaag	tagaaaatat	240
ggtttaattt	tattttttgtg	gtattttttat	agatgggtgt	tattttgttt	ggtagtgatg	300
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tggagttgaa	attttagttt	attttataga	agagaaaatt	gaggttttaga	gagattaggt	420
gttaagtttt	atgttaagta	tagggaagta	aaaataggat	atatttaata	taagggtggt	480
ttaggaatta	gtggttagga	tatgggagg	gtgttgagtg	tagatgggtg	gatgtaagt	540
gaggtgggtt	tttggagggt	tttggatttt	ttagaaaaga	ggatattagt	tgtttaggga	600
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taggggaggt	ttttgtatag	aagatttttt	atttgaggtt	gttttttagg	agtttagtta	960

## 47675-47.txt

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gaaggttgag	atggaaaagt	ttttgtagaa	taaggagt	tttagtagtt	tgaagaagg	1080
gaaggtgagg	ttgttttagat	gtgggttat	gtttttgttt	gttgggttag	gggtgggtgt	1140
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ggtagaaatg	ttataattta	tagggtagta	attggaatgt	ataaaagga	tttgttttat	2160
taagtgtggag	taagtagttt	aaagtgtatt	ttgttttagg	tttgattgat	atattataaa	2220
agtaagattt	gaaaggatta	aattgtttat	aagtatttta	attgattttg	agaataaagt	2280
ttaagaataa	gaatttttta	aatattaata	aaaaa			2315

&lt;210&gt; 463

&lt;211&gt; 2315

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 463

ttttttgttg	atgttttaaaa	aattttttatt	tttgagtttt	gttttttagaa	ttagttaaga	60
tattttgtgaa	tagtttgatt	tttttaggtt	ttgtttttat	gatgtattag	ttaggtttag	120
agtaataaatt	attttgggtt	atttatttta	tgtagtgag	gtgagttttt	tgtgtgtatt	180
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## 47675-47.txt

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gtttttgttt	ttttgtgttt	ggtagagaat	ttgatatttg	atttttttta	gttttagttt	1920
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aaatatattg	ttagtatagt	gtttgtagt	ttgggtatta	ttgttaagg	aggtgggtat	2040
tatttatgaa	gggtgttatag	agatggaggt	ggattatggt	ttttgttttg	gatttgtagt	2100
gggttggttt	gtttaaaggt	gtgaatatat	gtttttggta	ttggggggag	tgggggtttt	2160
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ttgatgggtg	tgttttttatt	tgaataattt	attttaggtg	ttatagggtg	gatgttaata	2280
atgtttgtgg	ttaggagatt	ttatgttagg	tatag			2315

&lt;210&gt; 464

&lt;211&gt; 2304

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 464

tgattttttt	gttttagttt	tttgagtagt	tagaattata	ggtgtgagat	atgtatttag	60
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aatttttgat	tttaagtgat	ttattttatt	ttgtttttta	aagtgttggg	attttaggag	180
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gtagtttttag	ttatttgggg	gggtgagggg	ggagaatgg	gtgaatttgg	gagggtggagt	2280
ttgtagttag	ttgagatggg	atta				2304

&lt;210&gt; 465

&lt;211&gt; 2304

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 465

tggtgttatt	ttggtttatt	gtaagttttg	tttttttaggt	ttatgttatt	tttttttttt	60
agtttttttga	gtagttggga	ttataggtgt	ttgttattat	gtttgttaaa	ttttttgtat	120
ttttagtaga	gatgggggtt	tattgtgtta	tttaggatgg	tttggattgt	ttgattttgt	180
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tgggtgtttg	tagtttttagt	tatttgggag	gttgaggtag	gagaatgggt	tgaatttttg	480
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tgggaggttg	aggtaagaga	attg				2304

&lt;210&gt; 466

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 466

gtggaatata	ttttttatat	agttatatta	tttaaaattt	attgttttta	agtttttatt	60
taaatgtttt	tttgtgaagt	tattttttgag	ttttttttta	aattgtagtt	ttttattttt	120
gtatttttta	ttgtttttta	tttttaattt	ttgttattgt	agttagaatt	atttgatata	180
tagtttttat	tttattgggt	ttttgttatg	ttttttttta	taagaatttg	aagtttatta	240
ggttgggagt	tttgtttgtt	gtgtttgttg	ttatttttta	gtgtttaaaa	ttgtttggta	300

## 47675-47.txt

tatagtaggt	atttaataat	ttttgaatta	gtgaaaatta	gatggtaggt	tggtagttttt	360
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aagggttatt	ataattatgt	gggggtttatt	ttgttttttt	ttgttttttt	tttttaggtt	480
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agagagtgaa	agaaggggaag	gattattttt	ttttttaaat	tggagtttaag	ggaggggaggt	660
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ggatttgttt						2470

&lt;210&gt; 467

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 467

ggatgagttt	attttaagtt	ggtgtgggga	aaaatataag	tatgtagatt	gattttattat	60
ataatgggag	ggggagggtta	ggttatgggt	agatttatta	taagggatga	agggtttgag	120
taatgttata	tgtagggtta	ttagtgtgtg	tgggtgggtg	ggggagttga	atattagtag	180
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gggtttttta	ttttttaggt	ttagagttta	tttgtgtgta	gtgtggtggg	ggtgttggtta	300
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ttggagtttt	gaggagggtg	gtttgggttt	tatgggtgtt	tatgttgatt	ggtttaagga	660
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## 47675-47.txt

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gaagtatttg	agtaaagatt	taaagatagt	gaattttgag	tagtgtggtt	atatggagga	2460
tgtgttttat						2470

&lt;210&gt; 468

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 468

tttaaaaagt	tttgtaaat	aaaagtttgg	gggtaaattt	atttggtagt	aaattttgat	60
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tttttgagag	ggagttttgt	tttgttgggt	aggttggagt	gtagagggtg	gattttgggt	420
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## 47675-47.txt

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ggtttggagg						2470

&lt;210&gt; 469

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 469

tttttgagtt	taggggttagg	ttgtgtattt	ttttaatttt	ttgtgtgagt	ttttttttgt	60
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## 47675-47.txt

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gttttttggg						2470

&lt;210&gt; 470

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 470

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gggtgtaggt						2470

&lt;210&gt; 471

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 471

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t t t t t g g t g t   t a a a g g t g g t   t g g t g t t g a g   g g t a g g a t t t   g t g t t t t t t t   g t a g g t t a g a   180
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a g t t t t t g t g   t t g t t t t g t t   t t a g t t t g t t   t t t g a t t g t t   t t t t t t t a t   t t t t t a g t t t   300
t g g a g t t g t g   a g t t t a g g t g   t t t g t t t a g t   g t t g t g t t t t   a g t t t t t g t t   t g t g g a g t t g   360
a a t t g t t t t t   t g g t t t t g g g   t t t t t a t t t t   g t a g t t g t t g   t a g t t t t g g g   t a t t g g t g t t   420
t g t t t t g g t t   g g t t t g g t g t   g g g t t t t g g t   t t t g t t g t g t   t t t t t t t g t g   g g t g t g t g a g   480
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t a t g g g t g t g   g g g a g g g g a g   g t g t t g t g g g   g a g t a g g t g g   t t g t g g g t g t   t a g t g t t t t t   600
t t a g t t t g g t   g t g t g g g a a g   g t a g t g t g g g   t t a t t a g a t t   g t g g t g g g g t   t a a g g t t t t t   660
t t t t g g t g t t   t t g t t t t t t t   a g t t g g t t g t   g t t t a a g t t g   g g g t t t t g g g   t g t g t t t t g t   720
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t t a t g t a g g g   t t g a a g t a g g   t t t g g t g t t a   g g g t t t t t t t   t g t g g g t t a g   a t t t g a t t g t   840
g a t t t t t g g t   a g a g t t g g g g   a t g t t g g t g t   t g a t t t t g t g   t t t g a t g t t t   g t g t t t t t t t   900
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g t t t a g g a g g   a g g g g a a t g g   t t g t t t t t t g   t t a a g t a g g g   t t t g t t a t t t   a t t t t t t t t g   1140
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a a t t t t a g t t   g g a g t t t g g g   t t t t a t g t t t   t t t t a g g g g t   t a t t t a g g a t   g t g a a t a t t t   1260
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g g g g a g t a g a   t t t g a g g t t g   g g t g t t t g g a   g g t a t g a g t a   g g a t t t t g g t   t g g t t t t t a g   1980
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t a t a a t a t a t   2470

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&lt;210&gt; 472

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 472

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g a g t a g t t g t   t t t t t t t a g t   t a t g g t a t g g   a t a t t t t a a t   t t g t t a t t t t   t t t t a t t a t t   120

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## 47675-47.txt

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&lt;210&gt; 473

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 473

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## 47675-47.txt

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&lt;210&gt; 474

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 474

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## 47675-47.txt

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&lt;210&gt; 475

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 475

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## 47675-47.txt

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&lt;210&gt; 476

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 476

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<210> 477  
 <211> 2470  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 477

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<210> 478  
 <211> 2470  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 478

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&lt;210&gt; 479

&lt;211&gt; 2470

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 479

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&lt;210&gt; 480

&lt;211&gt; 947

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 480

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&lt;210&gt; 481

&lt;211&gt; 947

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 481

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&lt;210&gt; 482

&lt;211&gt; 3120

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 482

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&lt;210&gt; 483

&lt;211&gt; 3120

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 483

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ttattttaaga	gatattgtta	ttgatttttg	aggggtttat	aaagttattg	ttatttttgt	420
ttaggaggag	gggagggtgtg	gtagggttgt	tattataagt	atgtataaag	atagggtttt	480
atattttaggt	aaaggaaaaa	aaagtttaat	gaaaaatatt	ttatattaag	agtatatTTT	540
atgagtattt	attaaaataga	aatatatTTa	gaagaaaggt	tatgttggtt	taatttaaaa	600
tattttgtata	ttgaattaat	atttttaata	tttatttagt	gttatttaaa	agtaattaaa	660
gagaaatata	aaattatttta	attatagggt	ttaaatatta	ggttttataa	aatttgttta	720
ttataatttt	tgataaaagta	atttatgttg	taaagtggtt	tgtttttgaa	tgatttaatt	780
aatggtataa	aagttttatt	aataaaaagaa	aagattttat	gttttatata	taattttatta	840
agatttttgta	taggattttat	gttaaattaa	agaatttgag	tattattttt	gtgatgtaga	900
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taaaaaatag	taattttttt	tttgttggtt	aggttgtatt	tataaattat	ggttttttgt	1020
aaatgggttt	ttatttttagt	atttgttgta	aggtttttta	tttttttgta	gttgtttttt	1080
tgggtttaata	gagggagaaa	aagtattgtt	gtagttgttt	gtaaaaagtt	attattattg	1140
tatttttgtaa	ttataaaaga	tattggtatt	tgtttgttat	tttgtttata	gggagtttat	1200
ttagatgttt	tataaaagta	ggtataattat	tgggtgattag	tattttatta	attaatatta	1260
ttatttatgt	ttttagtagt	ttgttttttt	tttttttttt	tatttttagt	agtttttttt	1320
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ttatttttgt	aaatgggtatt	ataaaaaata	aaattttttt	tggtttttaa	aatagttttt	1560
tttagtagat	tttttttaat	attttttatt	tttttttttt	gttaaaaaat	tttttttttt	1620
ttggatttga	agtataggaa	atttatTTTt	agtgtttagt	tggggggtgg	ggggatgatg	1680

## 47675-47.txt

tttttgtttt	tttgtttggt	atgtttagt	ggattttgtg	ttaaggaaa	gttattgggt	1740
taagatagt	tgaggga	ttatttttt	ttttttttt	gtagtaagt	tggttatggt	1800
ggttttagta	tttattttat	tttttagttt	ggtagtattt	ttattttgtt	tttagtgata	1860
ttttggtggg	ttgtattaat	atagttatat	gttggttatg	tgtgtttata	tttagttaat	1920
tggtgggttt	ttgatgggaa	tggggagtgt	tttggtttgt	atttagtgga	ttatatagtt	1980
gttttttttt	ttgttttttg	atttgtattt	tgtagtgggg	tatagggttt	tgtttaattg	2040
tatagtttgt	ttagtttttag	ttttgtattt	tttgggggta	tgtttttgtg	gttttagttt	2100
ttagtaagta	aggaagttag	tggtagttga	tatgtatagt	aaagagttag	gggaggtttg	2160
tagggtagaa	ggattagtat	aagatgttgg	aggtagggag	gagagtaata	aattatattt	2220
tgattgttat	gattttttgta	gtatagagaa	ataataatat	taaatgaatt	ataatgaata	2280
attttatatt	ataagttatt	gagttgttag	agtgatgatt	tgtagatag	attatttgaa	2340
ttatatttga	attaaaaata	gttattagaa	aagttatggt	agtagtttag	tagtggagat	2400
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taatttttagt	tattttaattt	tttagtggtt	taattttttg	ttgtataaaa	tgggaataga	2520
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atattgtatt	taaattgaat	taggtgatgt	ttaaattttt	aattttatga	ggttgaagga	2700
taaaatttaa	attgttaaga	atataataag	ttaaatttaa	attttggttt	aaatgggggg	2760
aaattagttt	ttattattta	tttaaaattt	aaaaggatat	tatatattta	gttttttttg	2820
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ttgggattat	aggtatgtgt	tattatgttt	agttaagttt	tgtattttta	gtagagatgg	3000
ggttttatta	tgttgggttag	gatggtttta	attttttgat	tttgtgattt	gtttattttg	3060
gtttttttta	gtattgggat	tatatgtgtg	agttattgtg	tttgggttaa	atttaaagtt	3120

&lt;210&gt; 484

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 484

gtgttttagt	ttttgaattt	atgttgtttg	ttattatatt	gtattgtttg	tggatgattt	60
atttgttttt	gtagttttat	ttgaagaatt	ttttttttt	tttttttgag	gtttgtttta	120
aatattattt	tttttgtgaa	gattgttttg	tgttttttag	gagagagtgt	gatttttttt	180
tttaggaatg	gtagtatttt	aaatatatat	atttttagt	atattttatt	ttgtatggta	240
atggtttgtt	tgtgagtttt	attttgtatt	agaggggtgag	ggttttgaag	atagtgttag	300
gttttattta	ttttgatatt	ttataatatt	tatttatatgt	ttgatgaatg	aatgtatagg	360
gggatgggtg	ggtgtatttt	ttttaatttt	tttaattttt	gaaataaata	ataaaatttt	420
attttttttt	agaagttttg	gtatgggttt	ttttattttg	ttatagttag	tgataattat	480
ttttattttt	tgtgtaattt	attaggtaag	aagtttatgt	agattttatt	ttagtatttt	540
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gatatagagt	tattatttta	agtatgatat	tttaatttta	aaagggtttt	tttgaagaat	660
attataaaat	tttttttttt	ttaagatttg	ttgggttagga	aagatgggag	aaaatgaatt	720
aatgtttata	tagaaaggag	gataatgggg	gtaaaaataa	tagatgaatg	tatgggtgga	780
tgagagaatg	gataaaatga	taggtgggata	tgttgatttt	ggatagatgg	gaaatgagt	840
gatatattaa	taaatagata	tgtgggtgga	tgggtggaga	agaggatggg	ggatggttgt	900
ggtttttatga	agagatgtga	aaaagggaagt	gtggaatgat	ggatgagaag	ttgtatggga	960
agatgaatag	aagaataggt	ggttgaataa	attaaaaggt	gtgtgggttg	atgaatgaat	1020
gagtgggatg	atagatggat	ttaagtgggt	agtggatgga	taggaggatg	gatggatgtg	1080
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atttgggtta	gttgagttaa	atgttagttt	tatgataggt	tatttagtagt	tttttttgag	1260
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agaatgagtt	tttttttttt	ttatttggaa	gatttattaa	ttttttattt	tttatttttt	1380
attgtgggta	tggagggtatt	gtgttattta	gggtaagatt	ttgttttttt	tttagttttt	1440
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gttatttttt	gttttgatgt	tgtgggttta	gtgggagaag	aaagtttagt	tgtttttggg	1620
tgtaggggtt	agtgggggtt	ggagggtatag	gtattttgtg	atatttttagg	ttttttgatt	1680

## 47675-47.txt

tatgtttttg	gtagttttga	ttattttatag	tttttagtaga	gtatgggggtg	ggggtagagg	1740
ggtttgtttg	ggaggggttg	tatttttttaa	aattttttgtg	ggttgttttag	ttatagtttt	1800
ttttgtttgg	gtgtgttttt	tgtttgtttt	ttttttttgt	tttaggttat	tgtttttaaat	1860
tttgaataaaa	aattgtagtt	aattttttgag	gtagttttat	tgtttagtgg	attttagttt	1920
ttgttaggtt	tggtttggtt	tttttgtttt	gttttttgtt	ggtttttgtt	ttgtgttttag	1980
ggatttttta	gttttttttg	tttgtgtttt	ttgtttgttt	tggatattat	ggataagttt	2040
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tgtttgttgt	agtttgggta	gtaagatggg	tgtgggggtg	ttagtgtgga	tttgggtgga	2160
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ttgggttttg	gaaagtaggg	ttgggatttg	tgttaaattg	ttggagaatg	tgtttgtgga	2280
agtattatatt	ggttgaaaga	aaaagagaaa	gagaagaaa	tttgttgggt	aggttgttgg	2340
tgtgtagttt	tgggtgaggt	tgtttagagtt	gtagtatatg	gtagaaaagta	attgtttttt	2400
tggatgtgta	tagttgttgt	ttggattaat	aggtttttgt	gtttaagggg	ttgttaagtt	2460
ttattggggt	gtgttttaggt	agggtagagt	tgggtggggg	a		2501

&lt;210&gt; 485

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 485

tgttttgttt	agttttgttt	tgtttagata	tagtttggtg	gggtttggtg	agtttttggg	60
tataggagtt	tgtttagttt	gataatgatt	gtgtgtattt	gggagaatgg	ttattttttg	120
ttatgtgttg	tagttttagt	gattttgttt	aaaattgtgt	gttggtagtt	tgtttaataa	180
attttttttt	tttttttttt	tttttttaaat	taaatgggtg	ttttatagat	atatttttta	240
atggtttagt	gtaaatttta	gttttgtttt	ttggagttta	atgtattttg	tagatagttt	300
atttgatttt	agtttttttag	ggttgattta	gttggagggg	ttgttgttgg	gtttgtgttg	360
agtattttgt	atttattttg	ttgttttaggt	tgtgggtggg	atttattgat	ttgtgttagg	420
tttagtggtg	tgaggttagag	tttttagggt	gtgtgttatt	aaaatttggt	tatggtgttt	480
ggagtgaatg	gaggggtgtg	gtgaaaggag	ttggaggatt	tttgggtgtg	gggtaggggt	540
tgggtggagga	tgggatgagg	atgggtggatt	gaatttggtg	gaggttgggg	tttgttgggt	600
aatgaggttg	ttttggaagt	tgggtgtagt	ttttatttga	ggttgaaaat	agtattttaa	660
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tttttttttg	ttggttttgt	ggtgttagga	tagaggatga	ttgaattgta	aaattttgta	960
ttattggggt	tagtttttgg	tttttttttt	tttgttaaat	ttttttgaat	ttggttggag	1020
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agggttttgt	ttgggtgggtg	tagtgttttt	gtgttttag	tggggagtga	gggggtgggga	1140
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ggtttgttat	aggggtgggtg	tttgggttag	ttaatattatg	tgtttaagtt	agtgtgaatg	1320
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tatttttttaa	tttattttaat	tattttatttt	tttattttatt	tttttatata	attttttatt	1560
tattattttta	tatttttttt	tttatatttt	tttataaaaat	tataattatt	tattatttttt	1620
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taagattaat	atattttatt	attattttat	ttattttttt	atttatttat	atgtttattt	1740
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ttgagattga	aatattatat	tttgaatgat	aaatttatat	ttattttata	tatatatttg	1920
tttaattgtt	ggaaattaaa	tgtagtattt	atttgttatg	tgaattatta	aaagtaaaatt	1980
tgtatggatt	ttttgttttg	tgagttatat	agaaaatgaa	agtgtattatt	agtggttgtg	2040
gtaagatgaa	aggaattatg	ttaggatttt	tgaggggaagg	taagattttg	ttattttattt	2100
taagaaattg	aaaagttgag	agaaatgtat	ttagttattt	ttttatgtat	ttattttatta	2160
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ttttattttt	tgggtatggaa	taaaattttat	aagtaggtta	ttattatgta	aggtgaaata	2280

## 47675-47.txt

tgttgtaaaa	tgtgtgtgtt	tgggggtgta	ttatTTTTtag	agaagggagt	tatattTTTT	2340
tttggagaat	attaggtagt	ttttataggg	gaagtgatat	ttgaggtagg	ttttaaagga	2400
aaaggaggag	gaattTTTT	gatgaaatta	tagggataag	taagttattt	ataggtagta	2460
tagtgtagt	atgaatagta	tggatttagg	agttagaata	t		2501

&lt;210&gt; 486

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 486

agatttattt	aaatttaaga	atgagaatat	aaatttatat	tttgaagtgt	tttatagaaa	60
ggtttatttt	aatgtttgga	gtatatattt	taatgaatat	ttatTTTTatt	ttatTTTTttt	120
ttatTTTTga	attaagtaat	tttgaattta	aagtTgttat	gattagtatt	gaaaagatta	180
ttggattatt	aattgtgtga	ttttgggata	gtaattTTTT	gtatTTTTtagt	ttgtttatat	240
gttatatatg	aaggttgaag	tttgattttg	ttttgtgatt	attatttttaa	atatttgatg	300
aaattaaatt	ttagtgtttg	gaatggtagt	ataataaaatt	tatttaagaat	aaataattta	360
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gataaataat	aaaagaaatg	aaagatgtat	atggtgagaa	ttgaaattat	tttgataagt	480
tttttattttg	tttattattt	aaaattaatg	attatgttga	atgtttataa	attataaaat	540
ataaaaagaaa	ttttataaat	gtgtatgtat	aggagtTTaa	gttattaaaa	gttttaaagt	600
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gtaggatttt	aaaaaatgta	ggtaattttt	ataaggggtt	ttggggagag	gtatatagag	2460
ggattttTggt	gttgaaaaag	atttagataa	aagaaattta	g		2501

&lt;210&gt; 487

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 487

ttggggttttt	tttgttttgaa	tttttttttaa	tattaagggtt	tttttgtatg	tttttttttta	60
aaagtttttta	tgaaagttat	ttgtatttttt	taagtgttta	tatttttttta	atttttgtttt	120
atagttttttt	gttttaatta	aagttttttta	ttaattgtttt	tttttttttta	agtttgtggg	180
tttttttttta	taagttttttt	gtttttgtttt	tttaagggggg	gaataaaaaga	aatgtgatta	240
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gtttggtgtt	gtttaggttg	gttatatagg	aagtgtggtg	gtttgggggaa	ggatgtggag	360
ggtgtgggat	ggtgttggaa	gatgtgggag	gatgtggggg	tgggttgaaga	ttttggttttt	420
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ggtttaagggt	ttttgttaatt	ttgaaagaga	tattgagaaa	atgagattttt	ttggggatttt	1140
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&lt;210&gt; 488

&lt;211&gt; 3000

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 488

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&lt;210&gt; 489

&lt;211&gt; 3000

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 489

gatatttagt	ttatgttata	taaaaattat	taaagtggga	ttgttgtagt	TgaaaagtTt	60
gattagtgtt	Tggagattag	aatgtgagtt	TTTaaagtat	atatgtattt	ggTattTggga	120
aagTTtaggt	gtTTgtgatt	TTTTgaagga	TTTTgggtTa	gtattTTTTgt	gtTTggatta	180
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## 47675-47.txt

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&lt;210&gt; 490

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 490

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&lt;210&gt; 491

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 491

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gtatggggag	atgaggttat	ggttgtgatt	ttgggtgggt	ttgggaagg	ggttgtaggt	1620
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tttgtgaaaa	ataagagttg	ttaaaatatg	gtttgataag	gttgaaaata	ttgaattttt	1980
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ggtttatatt	ttaatttttt	ggaatttgga	gataattgtt	gataattggg	attggtaggt	2460
tttatgtttt	tttagagaat	tgtaggatat	atggaagtga	t		2501

&lt;210&gt; 492

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 492

ggaggataga	aatataaatt	aaagaatgat	ataaataatt	ataaagttat	agttgttaaa	60
agaaaagtat	atggtgttaa	gagaatgtgt	aatataagat	ttatttatgg	agggtgaggga	120
aagtttgttt	attaaagaag	ttatgattta	atttatgaag	attaggaggt	ggttgggtga	180
agaaaaaaag	gttagaggaa	ggaagtttat	attggggaag	gttttaagta	taaagggtag	240
gaggattata	gaggtatatt	tatgaaattt	ggagaagggt	tttagtaagt	aaggagaagt	300
taaatgaaag	tttatgggag	agttggagggt	ttgaagatat	gtttaaggat	ttggttttta	360
tttttttttt	attttaagag	tagtgggaag	ttattaaatg	attttaatta	gagggttggt	420
ataattagtt	ttgtattttg	aaaagttgaa	tttagttttt	gtttgagaaa	ttgagtgaag	480
gagtttagaa	tggttgtggg	tgagggtgat	ttgtgggaga	tttttatata	agttatggta	540
gtggtatggg	ttggtgggat	aagagggaat	agggagaaga	tttggaattt	aatttttttt	600
tattgataaa	gttattttag	ttttggtaag	gttaattaat	ggtagggaaag	aagatgttta	660
gtttttttga	ttttattgta	ttttttgtat	ttttaatatg	agtattggga	agtggtaaaa	720
tatttagagg	tagtttgggt	gttaggtgga	gtatgagtta	aaattttagg	atgaagtaaa	780
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aatttattaa	atgaatttgt	agaagattta	ttgattagag	ggttgtatag	aattatattt	960
ttgagagtgg	gaagtagggt	gattatatag	tttattattt	aattaggata	tatttgaaag	1020
agaaaggggg	ttttattaat	atttaaatat	taaaatatgt	atattaggaa	tgttttgggt	1080
aaatttgggt	gttttagtaa	gaaaggaaat	ttgaaagttt	atattgtttt	gtttttatgt	1140
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aagtataagt	atttaggtga	tttttaattt	atttttaatt	ttatagtttt	tgttatatatt	1260
tatatatttt	gaaaaattta	ttttttatta	ttattatttt	gtgatagggt	attatttata	1320
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aagaaatgtg	tttgtgattt	aatagatttg	agtatttttt	tttggatttt	aatttghtaat	1680
ttgaaaatgt	attttttaag	tatttaggag	taatttgagg	aaagttgagg	ggagggtgga	1740

## 47675-47.txt

gatgttttga	tttattaggg	aaaatgtgga	tgttttttgt	tgttattttg	tgaattgtgt	1800
gtatttagtt	atTTTTgagt	aaatatTTtg	agtgaggaat	ttttgagtgg	tgtgggaggg	1860
tggtagaggg	tagttgaaa	ttggTTaaa	tttttgagg	ggttggTTta	ggaaatatga	1920
ttggtagtta	tgagagagtt	aggggttTga	tgttgaggag	agggagaagg	tttttgggtg	1980
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gggtgtTggg	ggTTTTttTg	gggtTTTTtT	ttttTgtTtT	t		2501

&lt;210&gt; 493

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 493

gtagtggaga	ggTggggatt	ttaggaagat	ttttTgtgtt	ttgttgagtt	tgggttgggg	60
attattttatt	TgatttttTga	atgtgtggTg	ggattTgtgtt	ggTgatattg	gtttatagga	120
Tgatgtgttag	TggTtatagg	ttttTgagta	gtttttTgatt	Tatggtagat	tttTgtgttt	180
gttatagatt	gagtttttag	Tgtagtggat	ggTgtttttt	TggattttTg	gttTgtgttt	240
TgtgtTgtgt	ttttttTgga	ttttTgtgtt	ggTtggtagt	gtagatgtgt	gggttagatg	300
TggTgtttTgt	ttgttagTta	ggaggggggt	TggaggtTgg	TgaggtTgtg	ggaggttttt	360
ggTggTtgag	ggaagTtTga	taggagTttTg	gtttttTgtt	TgagTgggtg	Tatgtgtggg	420
ggTgtTgtTg	ttttTgtgtg	gtgagTgatt	TattTaatTT	TaatTtagTg	ttTgtggggg	480
aataggaaat	ttttTgtTaa	TagtTgggtg	ggattttttt	ttgtttTgag	gttttttttt	540
ttttttTgat	gttttagTtt	TagtTttttt	gtagTtTgtt	attatgtttt	Ttagattagt	600
ttttttTgag	gtttTggTtTg	atTTtttagTt	gtttttTtatt	gtttttTtat	attatttagg	660
agTttttTgt	TttaagTatt	TattTaaGaa	TgattaaGtg	TatatagTtt	ataaagTaat	720
aatagaaaa	gtttatgttt	tttttagtag	attagaatat	ttgtTgtttt	tttttagTtt	780
tttttagatt	gtttttaggT	gttttagaga	TgtgtttTta	aatTgtaagt	Tgagatttag	840
gagTgaatat	TttaattTtat	TgagTtTgtg	TtatattTtt	ttttTaaata	aaatagTaat	900
ggTaaatttt	atTTttattaa	atTTgtgttt	TagtTgtgtt	Tatatgtatg	Tatgtatgtg	960
TatatattTgt	TaagtTgtaa	aatgtttttt	tttaggtTga	agTtttagagg	ttttttTtaa	1020
gtttTaatgt	atatattgat	TattTggaaa	ttttattTaa	aaatgtagat	TttaattTtag	1080
taggtTtagg	aggtaggtag	agattTttTga	ttttTaatga	gtattTggat	agagtgtttt	1140
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aaattTaaaag	TgggtTggga	gttattTtgaa	TgtTtTgtgt	tttattTttt	TaatgtaggT	1320
gaagaaagag	aatattTtatt	tttttatgtg	TaaatggggT	aatatgggag	TagaatagTa	1380
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TatatgtTtt	TgagtTtTaa	TattTaataga	atTTttTttt	ttttTtagat	atgtTttTgat	1500
Tggataataa	attatgtTat	TaattTattt	ttTattTttT	aagatattgat	ttTgtatagT	1560
ttttTggTta	TtagattTttt	TagTaatTta	ttTaatgaat	ttttTtattt	gagaggaaga	1620
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aaattTtaatt	ttTaaatttt	ttTgtTatt	TtaagtTgtt	attTgtTttT	ttttTggaatt	1740
TtaattTtatg	ttttattTtag	TattTaaGtt	gtttTtggat	gtttTgttat	ttttTtagtat	1800
TtatgtTgaa	gatTtagaaa	gtgtagTgaa	attaggaggT	Ttaggtattt	ttttTtttat	1860
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gtttTtttatg	agTtattTtt	agTtatggTt	gtttTgggtt	ttttTattTta	gtttTttTaaa	2040
TgagagTtTga	atTTtagTttt	Ttaaaatata	aaattagTta	TattTaatTtt	TtgattTaaaa	2100
TtattTtaattg	gtttTttTatt	gtttTtTgag	Taaagagaag	ataaaaaatta	gattTttTgaa	2160
TgtgtTttTta	agTttTttTaat	ttttTtTtaa	atTTttTattt	ggTttTttTtt	TgtTtattTga	2220
aagTttTtttt	TaaattTttgt	GaatattGttt	TtGtaattTtt	ttTattTttt	atgtTtagag	2280
ttttTttttag	TgtggattTtt	ttttTttTga	ttttTttTttt	ttTattTtagT	TaattTttTgg	2340

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tttttgtgga	ttgaattata	atttttttga	tgggtaagtt	tttttttatt	tttatgagta	2400
gattttgtat	tatatgtttt	tttggtatta	tatgtttttt	ttttaatagt	tgtaatttta	2460
taattatttg	tgttattttt	taatttatat	ttttattttt	t		2501

&lt;210&gt; 494

&lt;211&gt; 1508

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 494

attttataat	tataaatatt	tagtgtattt	tgtaaatatg	gtataatttg	ttggtataaa	60
attttattgt	tttagaaaat	attggtaata	aaattataga	aaagttaaag	atttttattt	120
tttattttga	agtgtttggg	agatatttta	gaaatggatg	ggaaatgta	aattttgtat	180
gtttgtttat	ttttttttt	atattgatta	gatgtaaatg	agtgttatta	aaagtatat	240
ataggatatt	atatagattt	tttttataag	ggattttata	agtttagatg	tgaaatgtat	300
tttaaagggt	ttttagttgt	tttttatttt	tttttttgtg	aaatagggaa	gatatatgtg	360
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ggattgtttt	aggatatggg	aggatagtgg	attgtttttg	ttgttttgtt	gtttatatgt	480
gggttttttg	taggtgtgtt	ggtttttttt	ttttttgttg	agaagaagtt	ttgttaaaat	540
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aaaataattt	gtttaagttt	tttagtgtgg	gagaaaatgt	tttatttgtt	ttttgttttt	660
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tatgtttggg	tttaattgtg	gtagaataaa	tttttgggtg	gtggttagga	agtttttttt	780
tagttattgt	tttttttttag	tgtttttttt	tttttttata	taataataaga	tttttttttt	840
ttagtttttt	ttaaagtatag	tttaaggaaa	tttttttata	gtttttattt	agttatgggt	900
tagtatgttt	gggggttaa	atgtagattt	ggaggtaggt	atttgtgggg	gggtgttggg	960
ttgggtgtgt	ggggaagtgt	ttgtttttgt	tattttgttt	tttaaataatt	ttgattgttg	1020
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gaatgggttt	gggtggggag	gtgaagagaa	gttaggaatg	ttttatgttt	ttttaatgga	1140
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attgggtttg	ttgggtttta	gatgtgtttt	ttggatgggt	ttaggatgtt	tttttgttgt	1260
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aggtgttatt	tatttgagtt	ttggggatag	tttttgggat	tttttgttaa	gtgtttaaaa	1380
tggtaggttt	ttaagtgggt	tgtggtgttt	ttgtattttt	taaaattttt	gaaatatttt	1440
aaaaattttt	ttgtattttg	gtgtttggta	gggtttttta	aaaaaagtaa	attttttttt	1500
taaatgta						1508

&lt;210&gt; 495

&lt;211&gt; 1508

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 495

tgtattttggg	agggaggttt	attttttttg	gaaaattttg	ttagatgtta	agatgtagaa	60
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atttgttgtt	ttgggtgttt	ggtggagagt	tttggggatt	gttttttaga	tttgggtaaa	180
taataatttt	tattttgtaa	tttatttttt	tgaattttta	gtaggtaggg	ttggattgtg	240
gggtttttgt	gataggggag	tatttttagat	ttatttagaa	aatgtgtttt	gggttttaata	300
gatttaattt	taggatttta	attttttttt	ttgtgttttt	ttagtttagg	ggtttttgag	360
gttttttttt	tattaggaaa	atataaaaata	tttttgggtt	ttttttattt	ttttgttttag	420
gtttattttt	tagtgggtgt	aatgttagag	gagtgttttg	aagtggaaaag	agggttgggg	480
ggtttagatta	gtagttggga	tattttggaga	ggtagatagt	agaagtggat	atttttttgt	540
atgtttgagt	tgggtgtttt	ttatggatgt	ttatttttga	gtttatgtat	ttgttttttag	600
atatgttggt	ttgtgggttg	atgaaaattg	tgaggagggt	tttttgggtt	gtgttttaag	660
ggaattgagg	aaggaagatt	ttgtattgta	tgggggggaaa	aaaagggtatt	ggaggggggt	720

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ggtggttggg	agggagtttt	ttggttggtt	attaaagggt	tgttttggtt	gtggttggtt	780
tggatgtgtg	ttgttttggg	tttaggtatt	ttttaagggt	ttggtagtag	agggtagggg	840
gtatttttgg	gggtagagag	tgagtaagaa	tgtttttttt	gtgttggggg	gtttggatag	900
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aggtggatgt	tttggtaggg	ttttttttta	gtaggggggg	agggagtga	tgtgtttgta	1020
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aagtaggtat	gtagaattta	atattttttt	tttgtttttg	aaatgttttt	taaatgtttt	1380
gaaataagaa	atgaagattt	ttaatttttt	tataattttt	ttattgatat	tttttggaat	1440
aatggaattt	tatgttaata	ggttatgtta	tatttgtaaa	atatattaaa	tgtttataat	1500
tgtagaat						1508

&lt;210&gt; 496

&lt;211&gt; 286

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 496

tttgtattag	gttggaagtg	gttggttagtt	ttttgtgtaa	ttttattttt	tggaaaagtg	60
gaattagttg	gtattgttta	gtgtgatttg	tgagggtgag	ttttaatagt	ttaaagaagt	120
aaatgggatg	ttatttttgt	ggggtttgtt	ttttgtgagg	tgttttattt	gtatttgtaa	180
tgtaaaatga	gggagtgtta	ggaaggaatt	tgttttgtaa	agttattggt	tttggttatt	240
agttttttatt	taatgttttt	gtgatgttgt	tgttgattta	tttggg		286

&lt;210&gt; 497

&lt;211&gt; 286

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 497

tttaaataga	ttagtagtag	tattatgaaa	gtattgggta	gaggttgatg	attaggatta	60
atggttttat	aagatggatt	ttttttta	gtttttttgt	tttgtatggt	agatatgggg	120
tgagtatttt	gtgaggagt	agttttgtgg	aggtgggtatt	ttatttggtt	ttttggattg	180
ttgggggtta	gttttataaa	ttatgttggg	taatgttagt	tgattttatt	tttttagaga	240
atggaattgt	atgggggatt	ggtggttatt	tttagtttag	tgtaaa		286

&lt;210&gt; 498

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 498

tttatatata	ttatgttttt	taaatgat	attagttttt	tgagggtaat	ttatattggt	60
aatagttttt	agatgtggaa	attgtgaaga	taatgttggg	gatgtggaag	taatataaat	120
tttggagt	tttagattta	ggtttgaatg	ttagattggt	ttttatttag	agtaatttta	180
gagtattatt	ttttatttta	attttttttt	aggttttttt	gtgtttatgt	gtttttttta	240
tttttgttta	ttgttttttt	agtgaatttt	gtattttttt	ttattgttag	tgtgtagata	300
tatagttttt	ttgggttttg	gatttatggt	aatttttttt	tattattttg	ttagtttatt	360

## 47675-47.txt

taatttttat	tgagtaatgt	tagttgaaa	ttgtggtggg	attaaatgtt	gtaatgagta	420
tttaaagtag	gttgaagtat	ttatgtat	tatttatata	tggtgaggta	tatttaagga	480
aggttgtagt	tattaaaatt	ttaggaaa	attttttatt	tttttaggtg	aaagggtttt	540
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tgtttatgat	gtttggttgt	gtataagata	aagttataat	aaagttataa	tttatttttt	840
ttttgtagaa	gattgtaaaa	agtaaaagag	atttaggtaa	aaatttttga	atgatttttg	900
gaatagagag	tttttttaga	attagaagt	aaaggaatt	aaaatatagg	gagggttagg	960
gtttttattg	atataaagga	aagatgtttt	ttttataggt	ttatgtttat	attttttttt	1020
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aagagtagtt	gtagtaatt	atattat	ttatgtttg	ttgtttatta	agaggtgaaa	1140
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aggtaatttg	gaaaagtaag	tggttgtata	taaagtaaat	gtttatagag	ttttggataa	1260
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&lt;210&gt; 499

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 499

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&lt;210&gt; 500

&lt;211&gt; 2448

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 500

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<211> 2448

<212> DNA

<213> Artificial Sequence

<220>

<223> chemically treated genomic DNA (Homo sapiens)

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 <211> 2344  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

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<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 503

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aggt
2344

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&lt;210&gt; 504

&lt;211&gt; 2350

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 504

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## 47675-47.txt

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gggtgttttg						2350

&lt;210&gt; 505

&lt;211&gt; 2350

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 505

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tatagggttg	ggttttgttt	gtgatatttg	tttatgtttg	aggagagagg	ttgggtgggt	240
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## 47675-47.txt

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agtgtatttg						2350

&lt;210&gt; 506

&lt;211&gt; 2206

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 506

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## 47675-47.txt

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&lt;210&gt; 507

&lt;211&gt; 2206

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 507

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&lt;210&gt; 508

&lt;211&gt; 2233

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 508

## 47675-47.txt

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&lt;210&gt; 509

&lt;211&gt; 2233

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 509

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&lt;210&gt; 510

&lt;211&gt; 2398

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 510

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&lt;210&gt; 511

&lt;211&gt; 2398

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 511

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 <211> 2114  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 512

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<210> 513  
 <211> 2114  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<400> 513

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tagaaataat	gtttttaatg	gatgggggtt	tagtaaaaata	ttaaatgtag	ttttttat	1020
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gtttttaaat	tttaggaaat	ttggagtata	ggttttaaat	tatttgggga	atttagagtt	1140
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aagagttatt	atttaatttt	tttttggttt	ttagaaagta	ttttttggtt	attttgtgtt	1260
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ttgagggggg	ttgagaatta	agggataaaa	tgtaatgtgt	ttttaaat	tagttttttt	2100
tttgttgtaa	tttt					2114

&lt;210&gt; 514

&lt;211&gt; 2382

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 514

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ttgagttggg	ggagaggggtg	gggggtggggg	tggttggggg	tttgaagt	aggttttttt	180
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ggatttttgg	attatagttt	ttattgtggg	tttagtttta	tttttagttt	attttgtggg	1320
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atgttttttt	agtttttttt	tagtgaagta	ggtgtgggtg	gg		2382

&lt;210&gt; 515

&lt;211&gt; 2382

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 515

ttttgttgtg	tttgttttgt	tgaggaggag	ttggaagaat	atgtttgtgt	gattttgggg	60
agtttttggg	gtagagtggg	gtgtgttggg	ggtattgggtg	gtgtgtagga	ttgggtgttt	120
ttttgagtg	ttttgttttg	ttgtagtgtg	ttaaaggttga	gatgagtggt	tgatattttt	180
attttttttt	ttttttagga	atttttttat	attttgatat	gtgttatggt	tttgtgtatt	240
ggaggaagta	ggtttttggg	gatttttggt	agtttgtaga	ggtttgggtg	ggtaatggga	300
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gtgaagtgtg	gttttggttg	atagtgggta	tttttagggg	tt		2382

&lt;210&gt; 516

&lt;211&gt; 2192

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 516

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aaagggtttt	atattttatta	tttttttaatt	tttgattttt	gtttggatag	aataattatt	360
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&lt;210&gt; 517

&lt;211&gt; 2192

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 517

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&lt;210&gt; 518

&lt;211&gt; 2192

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 518

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ttagtgttaa	ttataagtat	ttttattttt	ggttaggtaa	gttttgattt	ttgaattttg	540
agtttttaaat	tttttttaaa	atgagaaaga	gatgggtttt	tttagagttt	tttagttttt	600
aatttatagg	taaatattat	tgtatgttgt	tttttagtag	agattgggtt	taattaaagg	660
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## 47675-47.txt

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&lt;210&gt; 519

&lt;211&gt; 2192

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 519

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&lt;210&gt; 520

&lt;211&gt; 2244

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (2126, 2128, 2131, 2132)

&lt;223&gt; unknown base

&lt;400&gt; 520

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<210> 521  
 <211> 2244  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

<220>  
 <221> unsure  
 <222> (113, 114, 117, 119)  
 <223> unknown base

&lt;400&gt; 521

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 <211> 2420  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> chemically treated genomic DNA (Homo sapiens)

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&lt;210&gt; 523

&lt;211&gt; 2420

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 523

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## 47675-47.txt

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&lt;210&gt; 524

&lt;211&gt; 2344

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 524

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&lt;210&gt; 525

&lt;211&gt; 2344

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 525

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&lt;210&gt; 526

&lt;211&gt; 2366

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 526

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&lt;210&gt; 527

&lt;211&gt; 2366

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 527

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&lt;210&gt; 528

&lt;211&gt; 2264

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 528

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gattagtttg  gttaatatgg  taaaattttg  tttttattaa  aaatataaaa  attagttggg  60
tatggtgggt  tgtgtttgta  atttttagtt  tttgggaggt  tgaagtagga  gaattgtgtg  120
aattaggag  gtgtagggtg  tagtgggtta  gagattgtgt  tattgttttt  tagtttgggt  180
gatttttagg  ttaaattgtg  tttgatataa  aagtttttat  tgatatagat  tttttgatat  240

```

## 47675-47.txt

aaaagtttat	ttttttttga	taggtttgat	tgattttttt	ttttataaaag	ataaaagtgtg	300
ttttaataatt	taagaggaat	tatattgttg	tgaaaggggt	gttatgaatt	ttgggtttta	360
gtttttgtat	tatgttttag	tgtgatattg	gatttttaatt	tttttattat	taaagttaaat	420
gggttattat	aattttttta	gaagtagttt	taatttttaa	agataagatt	aaaaatatta	480
atagtgtttg	agtttttgga	attgaattag	tattggagtt	ttgtttgggt	gttttttttt	540
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gttttattag	tataattttt	tttattttgt	ttttatttgt	tgtagttttt	tttaagttta	720
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tgatgatatt	atttttattg	tttttttgag	gggttatttt	gaggtgtgtg	tagaggatta	1020
gaggattttat	tttaattttg	gtttgggtgat	agtgggaagt	gggaaaattg	gatgtaaaga	1080
gttttagatta	tggatttttt	ttgttaggaa	attagtattt	gtgaaggagt	gtagttttgt	1140
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tttaattttt	agagtaaagt	tgggggattg	ggagaaaatt	tagttttgtt	gtatggtggg	1260
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attaagagga	aagtttaaaag	agaggaagggt	gtttttttttg	aatgttttat	tttaaatgaat	1680
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tgttatattt	atttttttgt	ttttatgttg	tttggatgaa	ttattgtatt	ttttgtttta	1920
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gtgtgttttg	gtatagtaaa	gtttattttg	gttgggttgt	ggagttttgt	ttagttaggt	2100
gagtttaggg	gtgggtgtgt	gggtgtgtgt	gatgtgtgat	gttggatttt	ggaagtgtg	2160
tgtttgtggg	gaaaaggtag	ggaggggggtg	gtgttttttag	ttgggtttggg	gggtgtgttg	2220
tttggagatg	gaaagtttgg	gagtttgagt	aggttttggt	gtag		2264

&lt;210&gt; 529

&lt;211&gt; 2264

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 529

ttgtagttag	gtttgttttg	gtttttaaat	tttttgtttt	tgggtaattgt	attttttaaa	60
ttgggtgggg	atattatatt	ttttttgttt	tttttttatg	agtgtgttat	ttttgggatt	120
tgggtgttatg	tgttattgtg	tttttgtgtt	ttgtttttta	gtttattttag	ttgggtgggg	180
ttttgtggat	tagtgtagggt	ggatttttgt	gtgttgagat	atgttttttg	gggtatgggg	240
atagtgtttt	gtgggttttt	attatgtagg	tggaaaggga	taaatagggt	tatttgtatg	300
tgttttttga	agtgtgtttt	ttttttaaag	gttttgggag	tttttgggat	aggaaatatg	360
gtaatttgtt	taggtagtgt	agaaataaag	agataaatgt	aatgtgtatt	tatattttta	420
agttttattt	tttagtatgt	ataaattaaa	tatgtaatat	gtgtggggat	ttagtatat	480
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tgttttaagg	agatatattt	ttttttttag	gttttttttt	tagttttta	gagattaatt	660
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gttttttaaat	tgaagttagt	agttaatagt	tgtggatgaa	taaatgaatt	ggataaattt	840
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taatttttatg	tatatatagg	tttgtgggtt	tgggttagtt	gaaatatata	aggttgagtt	960
tttgtttgaa	agttttattg	ttttggggag	ttttatttta	tgatattgtt	atataatgga	1020
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## 47675-47.txt

tattgtatttt	ttagataggt	gaaaaggaaa	aagtatttta	gtaaaataaaa	ttgtgtttttt	1140
ttataagtgt	tggttttttt	gtgaggagga	tttatagttt	gagttttttt	tgtttaatttt	1200
ttttgttttt	tattgtttgt	aaattaaaagt	taaaatgagt	tttttagttt	tttatgtgtg	1260
ttttaaaatg	attttttagg	agagtggtag	gggtgatgtt	attgtttgtg	gatgatagga	1320
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tgatattgat	ggaattttag	aggaaattga	tggagatgta	gatgtggatt	tgaaagaagt	1560
tgtagtggag	gaaggtgagg	taggaagaga	tgtattgatg	aggttgaagg	aatatattaga	1620
gattgtgtta	ttggttgttt	gttgggtttt	tggttagttt	tttaataagt	atgagaattt	1680
gaatttatta	gaattttttt	tagattgtta	attttgaggg	ggaagagagg	ggatgattaa	1740
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attttttagt	gagatggggg	tttattatgt	tggttagggt	ggtt		2264

&lt;210&gt; 530

&lt;211&gt; 1334

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 530

gggtgtttttg	attttttttt	tattttgttt	ttttgggttt	tatttgtttg	tttttggttt	60
tttgtttttt	tttgtttttt	ggttttttag	agtttttttt	ttatggtagt	agtttttttg	120
gttttttggtg	tagttttttt	gtggatgatt	tttttgtttt	ggggttgagt	ttagttttttg	180
gatgttgttg	aaatttttga	gattatgtgt	gggtttgggt	gttgtttttt	tggtgggtgt	240
tattgttatt	gttgttgttt	ttgttgttgt	tgtttgtggg	atgttttagta	gtttgttgtt	300
tggttttttg	gattttgtgt	tttttggaa	ttgtttgttg	ttgttagagt	gtatgaatta	360
gttatgggtg	tgtgggagt	tttgtggtag	tgtagtagt	ggatattttg	tgagggtttt	420
tgttgggtgt	tgttgttgtt	tgttatgtta	tttattgtag	tttgtttggg	gaagtgttgt	480
gttttttttt	tttttttaag	tgattgttaa	atgtttattg	gttgggaattg	ttttggtaag	540
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ttagtttatt	ttagtgtgtt	tttttagttt	tttatttttt	attgttttag	atttttaata	660
ttatttattt	ttattttagag	aaataagggg	aattgttgta	ggtttggggg	tgaggggtgg	720
ttttgggatg	ggtagaaagt	gtaggtgtag	taggaaattt	ttgtatgttt	gtgtttatat	780
tggagtgttg	aggattttga	gaaatattaa	atgggatggt	tttttgggtt	tattgttttg	840
aaagagtatt	aatttttaggg	gaaatattga	aatagaagtt	ttgttattat	taaagaaaaa	900
agttttatta	ggatgaggaa	gaaataattt	tatgagaaag	aatgagttag	aaagtaataa	960
attaaatggt	gattgtaggg	gaattgttga	tttttggtaa	agggtttatg	aggttgtatt	1020
ggttttttgt	tgaagattag	gttatataga	tttttagagga	gttgggtttt	aatagaattt	1080
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tagtgggtgt	tgat					1334

&lt;210&gt; 531

&lt;211&gt; 1334

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 531

gttagtggttt	attgaagttg	ggttttggtt	ttggtttggt	tatatggagt	ttgattagtt	60
ttagttatgt	ttattttggg	ttgggagatt	tgtaaagtgt	tttttttttt	aatTTTTttg	120
tattatTTTTg	aagtttaggg	aagtaaagag	aggggtatat	ttggattgta	aaattaatgt	180
tttttggtgt	ttaggagaga	agggaaatgag	agagagagag	agatagagag	agagagagag	240
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tttaaatggga	gatttagtgt	atTTTtatgg	atTTTtgTta	ggaattagtg	atTTTtttTgt	360
agttattatt	tgattttattg	tttttttggt	tatttttttt	tataaagtta	tttttttttt	420
atTTtagtaa	gatttttttt	tttaatgatg	ataaagtttt	tgtttttagt	tttttttttag	480
gattgggtgtt	tttttaaaat	agtgaattta	gaaaattatt	ttgttttaata	tttttttaaaa	540
tttttgtagt	tttaatgtaa	gtgtaagtat	gtaaagggtt	tttgttatat	ttgtattttt	600
tgtttatttt	agaattattt	tttatttttg	ggtttgtaat	agtttttttt	gttttttttg	660
atagaggttg	gtgggtattag	gggttttagg	tagtaggagg	tgaggggttg	aggaggtgtg	720
ttagggtagg	ttgggttggt	ttggatatgt	gtgttttttt	gtggagttaa	agggttgggg	780
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agtagtagtt	agtaaaagt	tttgtaaagt	gttttagttg	tgtattgttg	tggggatttt	960
tatagtatta	tgattagttt	gtgtaatttt	gtagtagtaa	atgggttttg	aggaatatag	1020
gattgtgggg	gttgggtagt	gggttattga	gtattttgtg	gatgggtgga	gtagaggtgg	1080
tggtgggtgg	agtgggtatt	gggtggggaag	tagtagttaa	atTTgtgtat	gattttgaga	1140
gttttagtaa	tatttaggga	ttgggttttag	ttttggagt	agaggggtgt	ttgttgagaa	1200
gttgtgttg	agatgtggga	agtgtgtgtt	ataaggagg	agttttggga	agttggagga	1260
taggaggaga	tgggagttta	ggggtagatg	agtggagttt	gaggaggtag	ggtggaggga	1320
gagttaaggt	gttt					1334

&lt;210&gt; 532

&lt;211&gt; 2501

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 532

tttttgaaaa	ttgttaataa	aaattttggt	gttttatggg	ttagggggta	ttatggaatt	60
tgtggatatg	tgatgttttt	tttgatatt	tggttttaaa	atgttttttt	tttgattttt	120
ttttttttat	tttttaggtt	agttgatatt	tagggaaaa	aggaaaggat	ttatgtgaaa	180
tattgggggt	tgaatttttt	ttgataataa	gttttttaaa	aaagattttt	aaatgaaatt	240
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taaatttggt	tttttggtgt	ttgttgata	gaatatttta	ttatttaggt	attatgttga	360
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aaatgaagt	ttaaagatat	aatataaata	ttatTTtttt	ttttattata	aaaatttttt	660
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tgggaggaag	gagaggagta	gaaaagagaa	ttattgggta	tttggataaa	tatttgggtg	2160
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## 47675-47.txt

tatgtat	tttt	taaaatagaa	ataaaagtta	aaaaataaag	aaat	ttttatt	taaaagt	2280
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&lt;210&gt; 534

&lt;211&gt; 2327

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 534

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&lt;210&gt; 535

&lt;211&gt; 2327

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; chemically treated genomic DNA (Homo sapiens)

&lt;400&gt; 535

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&lt;210&gt; 536

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Detection primer for

&lt;400&gt; 536

ttggagttaa agtatttggt aaga

24

&lt;210&gt; 537

&lt;211&gt; 18

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Detection primer for

&lt;400&gt; 537

aaaaccacct tcaaaccc

18

&lt;210&gt; 538

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Detection primer for

&lt;400&gt; 538

atcctccaca ctcttcctct at

22

&lt;210&gt; 539

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Detection primer for

&lt;400&gt; 539

gaaattagggt ttggttttgt tt

22

&lt;210&gt; 540

&lt;211&gt; 19

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Detection primer for

&lt;400&gt; 540

gagattttgg gaggggtag

19

&lt;210&gt; 541

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Detection primer for

&lt;400&gt; 541

aactctatcc ttttccctct tc

22

&lt;210&gt; 542

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Detection primer for

&lt;400&gt; 542

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aactcactta actccaatcc c

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tttttagattg aggttttagg gt 22

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ccaacaacta aacaaaacct ct 22

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ataaataaca ccttccaccc ta

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ATCGTTTTGG GTTCGGGGAG GGAGAG

26

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AGTTTACGTG ATGGTGGCG

19

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CGCCACCATC ACGTAAAC

18

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CTCATAAACA AAACGTCTCG AA

22

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ACGTTATAGA GGAGGAGTTT ACGTG

25

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AGTTTCGCGG TTTATAGAGG TT

22

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GAACGACCAA AACTAACTCG AA

22

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GTCGCGTTGC GCGGTTTGAT TTAGTCGA

28

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AACGATAATA CGAACGACCA AA

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GTTTCGCGGT TTATAGAGGT TT

22

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GTAGTTTCGC GGTTTATAGA GG

22

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TAGTTTCGCG GTTTATAGAG GT

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ACGATAATAC GAACGACCAA AA

22

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CGATAATACG AACGACCAAA AC

22

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GGTAGTTTCG CGGTTTATAG AG

22

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GTTTAGGTAG TTTCGCGGTT TA

22

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GAACGATAAT ACGAACGACC AA

22

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ACGACCAAAA CTAACGCGAA AT

22

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TTTCGCGGTT TATAGAGGTT T

21

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TAGTAGGGTT TCGATTTTCG G

21

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TACACCTATC TATAAACGCG CC

22

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TGGTGGTTGG TGGAACGTGC GATTGT

26

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AGGTAGTTTC GCGGTTTATA GAG

23

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<400> 1204  
AACGACCAAA ACTAACTCGA AAT

23

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TTAGTAGGGT TTCGATTTTC GG

22

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TTCGCGGTTT ATAGAGGTTT C

21

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CGATAATACG AACGACCAAA A

21

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CGGTTTATAG AGGTTTCGGT TC

22

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GTTTCGCGGT TTATAGAGGT T

21

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<400> 1210  
AACGATAATA CGAACGACCA A

21

<210> 1211  
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<400> 1211  
TTTAGGTAGT TTCGCGGTTT AT

22

<210> 1212  
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<400> 1212  
GAACGATAAT ACGAACGACC A

21

<210> 1213  
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<400> 1213  
GTTTATAGAG GTTTCGGTTC GC

22

<210> 1214  
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<400> 1214  
TTTCGCGGTT TATAGAGGTT TC

22

<210> 1215  
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<400> 1215

ACGATAATAC GAACGACCAA A

21

<210> 1216

<211> 22

<212> DNA

<213> Artificial Sequence

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<400> 1216

TTAGTTTTGT ATCGTTCGGG TT

22

<210> 1217

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<212> DNA

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<400> 1217

TCCCTCGATA AATCAAAAAC G

21

<210> 1218

<211> 47

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1218

ATCGTTTTGG CGCGTTTATA GATAGGTGTA TGAAGATTTT TACGATT

47

<210> 1219

<211> 21

<212> DNA

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1219

TTTATAGAGG TTTCGGTTCG C

21

<210> 1220

<211> 22  
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<400> 1220  
TACGAACGAC CAAAAC TAAC TC

22

<210> 1221  
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<400> 1221  
AGTAGGGTTT CGATTTTCGG

20

<210> 1222  
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<400> 1222  
ACACCTATCT ATAAACGCGC C

21

<210> 1223  
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<400> 1223  
TAGGTAGTTT CGCGTTTAT AGAG

24

<210> 1224  
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<400> 1224  
AATACGAACG ACCAAA ACTA ACTC

24

<210> 1225  
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<400> 1225  
GCGGTTTATA GAGGTTTCGG T

21

<210> 1226  
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<400> 1226  
AACTAACTCG AAATCCCGAA CT

22

<210> 1227  
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ATACACCTAT CTATAAACGC GCC

23

<210> 1228  
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<400> 1228  
ACCTATCTAT AAACGCGCCA A

21

<210> 1229  
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<400> 1229  
ATACGAACGA CCAAACTAA CTC

23

<210> 1230  
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<400> 1230  
AGTTTCGCGG TTTATAGAGG T

21

<210> 1231  
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<400> 1231  
AATACGAACG ACCAAACTA ACT

23

<210> 1232  
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<400> 1232  
CGAACGACCA AACTA ACTC G

21

<210> 1233  
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<400> 1233  
CCTATCTATA AACGCGCCAA

20

<210> 1234  
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<400> 1234

GATTTTCGAGT TAGTTTTGGT CG

22

<210> 1235  
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<400> 1235  
TAACTTCTAT TACCCGCGCC

20

<210> 1236  
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<400> 1236  
TTCGCGCGGG CGTATCGAGT TCGG

24

<210> 1237  
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<400> 1237  
CGAAATCCCG AACTACAAAA

20

<210> 1238  
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<400> 1238  
GGGGGAGTCG CGTTGCGCGG

20

<210> 1239  
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
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CGACCAAAAC TAACTCGAAA T

21

<210> 1240  
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<400> 1240  
TTAGGTAGTT TCGCGGTTTA TAG

23

<210> 1241  
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<400> 1241  
AGGTTTAGGT AGTTTCGCGG

20

<210> 1242  
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<400> 1242  
CGTTAAGAGG CGAGGAAATA A

21

<210> 1243  
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<400> 1243  
CCTAACTCGA CGCAACTAAC C

21

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<400> 1244  
AACGGCGGGC GGGACGAACG ATTAG

25

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<400> 1245  
GCGGTTTATA GAGGTTTCGG

20

<210> 1246  
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<400> 1246  
TAACTCGAAA TCCCGAACTA CA

22

<210> 1247  
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<400> 1247  
TCGCGGTTTA TAGAGGTTTC

20

<210> 1248  
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<400> 1248  
CCCTAACTCG ACGCAACTAA

20

<210> 1249  
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<212> DNA  
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<400> 1249  
GGTTTAGGTA GTTTCGCGGT

20

<210> 1250  
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<400> 1250  
TCGAAATCCC GAACTACAAA A

21

<210> 1251  
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<400> 1251  
GATAATACGA ACGACCAAAA CT

22

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<400> 1252  
TAGTCGAGGT TTTGTAGTTC GG

22

<210> 1253  
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ACGAACGACC AAAACTAACT C

21

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<400> 1254  
GATAATACGA ACGACCAAAA CTAA

24

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<400> 1255  
TAATACGAAC GACCAAAACT AACTC

25

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<400> 1256  
CACCTATCTA TAAACGCGCC

20

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<400> 1257  
TTCGCGGTTT ATAGAGGTTT

20

<210> 1258  
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<400> 1258  
CGGTTTATAG AGGTTTCGGT T

21

<210> 1259  
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 <400> 1259  
 CTCGAAATCC CGAACTACAA

20

<210> 1260  
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 <400> 1260  
 CGTTAAGAGG CGAGGAAATA AG

22

<210> 1261  
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 <400> 1261  
 CTAACCGAAA ACGCGAAACT A

21

<210> 1262  
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 <400> 1262  
 GTCGGGAGAG GGGCGCGGAA CGG

23

<210> 1263  
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 <400> 1263  
 TAACCGAAAA CGCGAAACTA

20

<210> 1264  
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<400> 1264  
GTTCTGGGATT TCGAGTTAGT TT

22

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<400> 1265  
TTATAGAGGT TTCGGTTCGC

20

<210> 1266  
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<400> 1266  
TTTAGGTAGT TTCGCGGTTT A

21

<210> 1267  
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<400> 1267  
GAGTTAGTGC GTTCGATTGT G

21

<210> 1268  
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<400> 1268  
GCCTACTCTA CTTCCGCGAT

20

<210> 1269  
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<400> 1269  
GGGGGCGGAA AGAGGCGCGT T

21

<210> 1270  
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<400> 1270  
TAATACGAAC GACCAAACT AACT

24

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<400> 1271  
ATAAAGGTTT CGTTAAGAGG CG

22

<210> 1272  
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<400> 1272  
ATACCCCTAA CTCGACGCA

19

<210> 1273  
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<400> 1273  
TTTAGTTTTG TATCGTTCGG GTT

23

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<400> 1274  
CGCGGTTTAT AGAGGTTTCG

20

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<400> 1275  
GTCGGAAGGT GCGAGTTAGT

20

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<400> 1276  
GATCGACCGA ATAAAAACAA AT

22

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<400> 1277  
CGTTTTTAGA TCGTGATTTT CG

22

<210> 1278  
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20

<210> 1279

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<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1279

AAGTTTTCGCG GCGGGGCGC GT

22

<210> 1280

<211> 22

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<400> 1280

ATTTTCGAGTT AGTTTGGTC GT

22

<210> 1281

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1281

TCGGAAGGTG CGAGTTAGT

19

<210> 1282

<211> 19

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1282

GTAGGGTTTC GATTTTCGG

19

<210> 1283

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<400> 1283  
AACTTCTATT ACCCGCGCC

19

<210> 1284  
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ATCGTTCGCG CGGGCGTATC GAG

23

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CAATCGAACG CACTAACTCG

20

<210> 1286  
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ACCGAAAACG CGAAACTAAT

20

<210> 1287  
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AAGGTGCGAG TTAGTGCGT

19

<210> 1288  
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AGGTGCGAGT TAGTGC GTT

19

<210> 1289  
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<400> 1289  
TAGTTTTGTA TCGTTCGGGT T

21

<210> 1290  
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<400> 1290  
CTCGATAAAT CAAAAACGAA AA

22

<210> 1291  
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<400> 1291  
ACGCACTAAC TCGCACCTT

19

<210> 1292  
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<400> 1292  
AACGCACTAA CTCGCACCT

19

<210> 1293  
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<400> 1293  
TCGGGATTTT CAGTTAGTTT T

21

<210> 1294  
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<400> 1294  
TTCGGGATTT CGAGTTAGTT T

21

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TCGTTAAGAG GCGAGGAAAT A

21

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<400> 1296  
AGTTTCGCGG TTTATAGAGG TTT

23

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<400> 1297

AACCGCGATA CCCCTAACT

19

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AACTCGAAAT CCCGAACTAC A

21

<210> 1299  
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CGGTTTATAG AGGTTTCGGT

20

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GAAACTAATC GTTCGTCCCG

20

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CGGGAAAGTA GCGGTAAGT

19

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
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CCGAACTACA AAACCTCGAC TA

22

<210> 1303  
<211> 28  
<212> DNA  
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<400> 1303  
AGATGCGAGG TTTAGGTAGT TTCGCGGT

28

<210> 1304  
<211> 20  
<212> DNA  
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<400> 1304  
AGGCGAGGAA ATAAGAGTCG

20

<210> 1305  
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<212> DNA  
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<400> 1305  
CCCTAACTCG ACGCAACTAA C

21

<210> 1306  
<211> 23  
<212> DNA  
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<400> 1306  
TTTAGTCGAG GTTTTGTAGT TCG

23

<210> 1307  
<211> 33  
<212> DNA  
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<220> bisulfite treated  
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
<400> 1307  
TAGTTTTGGT CGTTCGTATT ATCGTTCGCG CGG

33

<210> 1308  
<211> 20  
<212> DNA  
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<400> 1308  
AGGTTTCGTT AAGAGGCGAG

20

<210> 1309  
<211> 21  
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<400> 1309  
ACTAACTCGA AATCCCGAAC T

21

<210> 1310  
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<212> DNA  
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<400> 1310  
AGTTCGGGAT TTCGAGTTAG TT

22

<210> 1311  
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<400> 1311  
ACTTCTATTA CCCGCGCC

18

<210> 1312  
<211> 21

<212> DNA  
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<400> 1312  
AGTCGAGGTT TTGTAGTTCG G

21

<210> 1313  
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<400> 1313  
CCGAAAACGC GAAACTAAT

19

<210> 1314  
<211> 25  
<212> DNA  
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<400> 1314  
ATAATACGAA CGACCAAAC TAACT

25

<210> 1315  
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<212> DNA  
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<400> 1315  
GCGATACCCC TAACTCGAC

19

<210> 1316  
<211> 20  
<212> DNA  
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<400> 1316  
ACGATAATAC GAACGACCAA

20

<210> 1317  
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<400> 1317  
ACTCGAAATC CCGAACTACA A

21

<210> 1318  
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<400> 1318  
ACTAACCGAA AACGCGAAAC

20

<210> 1319  
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<400> 1319  
TCGAAATCCC GAACTACAAA

20

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<212> DNA  
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<400> 1320  
GGGTCGTTTT TAGATCGTGA TT

22

<210> 1321  
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<400> 1321  
CGGGTCGTTT TTAGATCGT

19

<210> 1322  
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<212> DNA  
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<220> bisulfite treated  
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<400> 1322  
CACAAATCGAA CGCACTAACT C

21

<210> 1323  
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<220> bisulfite treated  
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<400> 1323  
TAAAGGTTTC GTTAAGAGGC G

21

<210> 1324  
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<400> 1324  
TGTTTCGGTTT CGGAGGTAG

19

<210> 1325  
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<212> DNA  
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<400> 1325  
ATCACGATCT AAAAACGACC C

21

<210> 1326  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220> bisulfite treated  
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<400> 1326  
GGCGTTGGAG CGGTTAGTGG TAGTCGGGTA CGG

33

<210> 1327  
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<212> DNA  
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
<400> 1327  
CGGTTTGATT TAGTCGAGGT TT

22

<210> 1328  
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<400> 1328  
TTCGGGATTT CGAGTTAGTT TTGGTCGTTC GTATTATCGT

40

<210> 1329  
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<400> 1329  
AAGGTTTCGT TAAGAGGCGA G

21

<210> 1330  
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<400> 1330  
CTAACTCGCA CCTTCCGAC

19

<210> 1331  
<211> 20  
<212> DNA  
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<400> 1331  
TCGTTAAGAG GCGAGGAAAT

20

<210> 1332  
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<212> DNA  
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<400> 1332  
ATCGAACGCA CTAACTCGC

19

<210> 1333  
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<400> 1333  
GTTTTGTAGT TCGGGATTTC G

21

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<400> 1334  
AACTAACCGA AAACGCGAA

19

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<212> DNA  
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<400> 1335  
AACCGAAAAC GCGAAACTA

19

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<400> 1336  
TCTCGAAAAA CTCTCCTAAA CG

22

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<400> 1337  
CGTAAAGTTC GGTTTAATTT GG

22

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<400> 1338  
AGGCGTTATC GTCGGCGTAT TTCGGA

26

<210> 1339  
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<400> 1339  
CTCGAAAAAC TCTCCTAAAC GA

22

<210> 1340  
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<400> 1340  
TCGAAAAACT CTCCTAAACG AC

22

<210> 1341  
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1341

ACACCGCGAC TATTATACGA CT

22

<210> 1342

<211> 22

<212> DNA

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<400> 1342

TTCGGTTGTT ATTGATCGTT TT

22

<210> 1343

<211> 33

<212> DNA

<213> Artificial Sequence

<220> bisulfite treated

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<400> 1343

ATTCGTTAGT TGTTTTCGGA GTCGAGTAGG TTT

33

<210> 1344

<211> 22

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1344

GAGTTAGTTA GGTTCGTAGG CG

22

<210> 1345

<211> 31

<212> DNA

<213> Artificial Sequence

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<400> 1345

ATCGTCGGCG TATTTCCGAG AAGAAGTTTT T

31

<210> 1346

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<212> DNA  
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<220> bisulfite treated  
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<400> 1346  
TCGTGTTTCGG TTGTTATTGA T

21

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<400> 1347  
GTTCGTGTTC GGTGTTATT G

21

<210> 1348  
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<400> 1348  
CGAAAAACTC TCCTAAACGA CT

22

<210> 1349  
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<400> 1349  
GAGTTAGTTA GGTTCGTAGG CGT

23

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<400> 1350  
CACCGCGACT ATTATACGAC TA

22

<210> 1351  
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<400> 1351  
TTCGTGTTTCG GTTGTTATTG AT

22

<210> 1352  
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<400> 1352  
TGTTTCGGTTG TTATTGATCG TT

22

<210> 1353  
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<400> 1353  
CTTAATAAAA CCTCGATTTC CG

22

<210> 1354  
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<400> 1354  
TTTGTTTATA GGCGCGTTAG AG

22

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<400> 1355  
GGTTCGAGCG GTGTAGGGTT GGGT

24

<210> 1356  
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<400> 1356  
TTCGTAAAGT TCGGTTTAAT TTG

23

<210> 1357  
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<400> 1357  
AAACACCGCG ACTATTATAC GA

22

<210> 1358  
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<400> 1358  
AGTTAGTTAG GTTCGTAGGC GT

22

<210> 1359  
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<400> 1359  
ACTTAATAAA ACCTCGATTT CCG

23

<210> 1360  
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<400> 1360

GTGTTTCGGTT GTTATTGATC G

21

<210> 1361  
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<400> 1361  
TAAAACGATC AATAACAACC GA

22

<210> 1362  
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<212> DNA  
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<400> 1362  
GTATTTTTCG GTAAGAGACG GC

22

<210> 1363  
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<400> 1363  
TTCGGGAGTT ACGGTTTGAG GGCGATTTCGG GCGA

34

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
<400> 1364  
TCGTAAAGTT CGGTTTAATT TG

22

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<400> 1365  
TCGGTTGTTA TTGATCGTTT T

21

<210> 1366  
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<400> 1366  
ACACCGCGAC TATTATACGA CTA

23

<210> 1367  
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<400> 1367  
CGAACGACCA ATAAAAACAT AA

22

<210> 1368  
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<212> DNA  
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GCGTATTTTCG GAGAAGAAGT TT

22

<210> 1369  
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<400> 1369  
TTCGAGAAAG ATGTCGTCGT TTTAATAGTA ATTTATTTTC GT

42

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<400> 1370  
TATTTTTCGG TAAGAGACGG C

21

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<400> 1371  
AATTTATCCT TACCCGACCG

20

<210> 1372  
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<212> DNA  
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<400> 1372  
TCGTTTAGGA GAGTTTTTCG AG

22

<210> 1373  
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<212> DNA  
<213> Artificial Sequence

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<400> 1373  
AAAGATGTCG TCGTTTTAAT AGTAATTTAT TTTCGTTTCG GAG

43

<210> 1374  
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<220> bisulfite treated  
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<400> 1374  
ACGAACGACC AATAAAAACA TAA

23

<210> 1375  
<211> 22

<212> DNA  
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TTAAAACGAT CAATAACAAC CG

22

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ATTTATCCTT ACCCGACCGA C

21

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TATTTGTTTA TAGGCGCGTT AG

22

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GTTTCGTTGT TATTGATCGT TTT

23

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GTTAAAACGA TCAATAACAA CCG

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TTGTTTATAG GCGCGTTAGA G

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ACTCTAATCG CTCGCACTAC C

21

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ATTAAGTTTT ATGGTCGCGG TT

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AGGGTGAGTC GAGATTTAGA CGTAGATTTT TTAATTTTGT T

41

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<400> 1384  
AACACCGCGA CTATTATACG A

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CCGCGACTAT TATACGACTA AA

22

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GACCAACTAC GTCGAACCAA

20

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GTTTCGGTTGT TATTGATCGT TT

22

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CGTTCGTGTT CGGTTGTTAT

20

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AACACGCGA CTATTATACG ACT

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ACGAACCTAA CCGTAAAAAC G

21

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TTGGGTCGTA AGTTTTATAG TCG

23

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TACGTTTTAT TAATTATTAG TCGGGGTTTCG GAAGCGCGTT CGG

43

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ACCGCGACTA TTATACGACT AAA

23

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<400> 1394  
ACCAACTACG TCGAACCAAA

20

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TCGGTTGTTA TTGATCGTTT TA

22

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ATTTGTTTAT AGGCGCGTTA GAG

23

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AATTAAGTTT TATGGTCGCG G

21

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CAACTCTAAT CGCTCGCACT AC

22

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TAAGTTTTAT GGTTCGCGGT T

21

<210> 1400  
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<400> 1400  
TTAAGTTTTA TGGTCGCGGT T

21

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<400> 1401  
TATATTGTT TATAGGCGCG TT

22

<210> 1402  
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<400> 1402  
AAAACGATCA ATAACAACCG A

21

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ATATTTGTTT ATAGGCGCGT TAG

23

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<400> 1404

ATTTTTCGGT AAGAGACGGC

20

<210> 1405

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AACCGAACT TTACAACCCG

20

<210> 1406

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<212> DNA

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<400> 1406

ATTTTGTGTTG TTCGCGTTAA GT

22

<210> 1407

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GGTGCGTTTCG CGCGGACGGT

20

<210> 1408

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TTTATCCTTA CCCGACCGAC

20

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TGGGTCGTAA GTTTTATAGT CG

22

<210> 1410  
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<400> 1410  
GTTTTATGGT CGCGGTTTTT AG

22

<210> 1411  
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<400> 1411  
TTCGCGTTAA GTCGGGTTTCG GTGCGT

26

<210> 1412  
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<212> DNA  
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<400> 1412  
AGTTTTATGG TCGCGGTTTT TA

22

<210> 1413  
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<400> 1413  
AACGAACGAC CAATAAAAAC AT

22

<210> 1414  
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<400> 1414  
GGCGTATTTTC GGAGAAGAAG T

21

<210> 1415  
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<400> 1415  
AGTCGTTTAG GAGAGTTTTT CG

22

<210> 1416  
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ACTCGACTCC GAAAACAACT AA

22

<210> 1417  
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GCGTTTCGTT CGTAAGGTT

19

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<400> 1418  
TTCGTGTTTCG GTTGTTATTG ATCGTTTTAA CGTCGTTAGG GACGA

45

<210> 1419  
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<400> 1419  
CTCTAATCGC TCGCACTACC

20

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<400> 1420  
TCCGAACCCC GACTAATAA

19

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TAGTAATTTT GTTTCGGGTC GT

22

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CTCGAACCAA CTCTAATCGC T

21

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CGACTATAAA ACTTACGACC CAA

23

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<400> 1424  
CGGAAAAGCG GTGTTTATTA G

21

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<400> 1425  
AGCGGTGAAG GGTTCGAGCG GTG

23

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TTTATGGTCG CGGTTTTTAG

20

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TTTTATGGTC GCGGTTTTTA G

21

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AATTTTGTGTT GTTCGCGTTA AG

22

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<400> 1429  
CTACTCGACT CCGAAAACAA CT

22

<210> 1430  
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TGTTTATAGG CGCGTTAGAG C

21

<210> 1431  
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<400> 1431  
ACCGAACTT TACAACCG

19

<210> 1432  
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<400> 1432  
GAATTAAGTT TTATGGTCGC GG

22

<210> 1433  
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<400> 1433  
TTAAGTTTTA TGGTCGCGGT TT

22

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<400> 1434  
GGGTGAGTCG AGATTTAGAC GTAGATTTTT TAATTTTTGT T

41

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<400> 1435  
TTAATAAAAC CTCGATTTC G

21

<210> 1436  
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<400> 1436  
CGAAACCTCG AACCAACTCT A

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<400> 1437  
AAACTTCTTC TCCGAAATAC GC

22

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<212> DNA  
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<400> 1438  
GTCGAGGTTT TTGTAGGTCG

20

<210> 1439  
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<400> 1439  
TTTGGAGTTA GTTAGGTTTCG TAGGCGTTAT CGT

33

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<400> 1440  
AACTCTAATC GCTCGCACTA CC

22

<210> 1441  
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<400> 1441  
CGTTCGTGTT CGGTTGTTAT T

21

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<400> 1442  
TTCCGAACCC CGACTAATAA

20

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<400> 1443  
AGTTAGTTAG GTTCGTAGGC G

21

<210> 1444  
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<400> 1444  
AACCTAACCG TAAAAACGCC

20

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<400> 1445  
GTCGTTTAGG AGAGTTTTTC GAG

23

<210> 1446  
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<400> 1446  
TTATCCTTAC CCGACCGAC

19

<210> 1447  
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<400> 1447  
AACTTAATAA AACCTCGATT TCCG

24

<210> 1448  
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<400> 1448  
TTTTTCGGTA AGAGACGGC

19

<210> 1449  
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<400> 1449  
TCGAAAAACT CTCCTAAACG A

21

<210> 1450  
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<400> 1450  
TCGGGAGTTA CGGTTTGAG

19

<210> 1451  
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<400> 1451  
ACGACTATAA AACTTACGAC CCA

23

<210> 1452  
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<400> 1452  
GGTGAAGGGT TCGAGCGGTG TAGGG

25

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<400> 1453  
GGAGTTACGG TTTGAGGGCG ATTCGGG

27

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<400> 1454  
CGAAAACTC TCCTAACGA C

21

<210> 1455  
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<400> 1455  
AAGTTTATG GTCGCGGTTT

20

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<400> 1456  
CGACCTACAA AAACCTCGAC

20

<210> 1457  
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<400> 1457  
GTAGTGCGAG CGATTAGAGT TG

22

<210> 1458  
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<400> 1458  
TTCGGTTGGG TTAAGTCGCG TAGCGCGG

28

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<400> 1459  
ACCAACTACG TCGAACCAAA A

21

<210> 1460  
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<400> 1460  
CCGAAATCGC AATCTAAAAC T

21

<210> 1461  
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<400> 1461  
AAAACACCGC GACTATTATA CGA

23

<210> 1462  
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<400> 1462  
GTTTATAGGC GCGTTAGAGC

20

<210> 1463  
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<400> 1463  
TTTTGTTGTT CGCGTTAAGT C

21

<210> 1464  
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<400> 1464  
AACTTCTTCT CCGAAATACG C

21

<210> 1465  
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<400> 1465  
TAGTGCGAGC GATTAGAGTT G

21

<210> 1466  
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<400> 1466  
CCGAAACTTT ACAACCCGA

19

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1467

CGTTATCGTC GCGTATTT

19

<210> 1468

<211> 20

<212> DNA

<213> Artificial Sequence

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TTTTATTCCC CGCTAACGAC

20

<210> 1469

<211> 22

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1469

TTTCGGTAAA TTCGTAGGTT TT

22

<210> 1470

<211> 37

<212> DNA

<213> Artificial Sequence

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<400> 1470

TACGATGGCG GGGCGGCGGC GCGAATATAT AAAGGAG

37

<210> 1471

<211> 20

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1471

CCAACCTACGT CGAACCAAAA

20

<210> 1472

<211> 20  
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
<400> 1472  
CGTGTTTCGGT TGTTATTGAT

20

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<400> 1473  
CGAACCTAAC CGTAAAACG

20

<210> 1474  
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<400> 1474  
CCAAATTAAA CCGAACTTTA CG

22

<210> 1475  
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<400> 1475  
TTGGGTTAAG TCGCGTAGC

19

<210> 1476  
<211> 48  
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<400> 1476  
GTCGCGAAGT TGTTTAAATT TCGTATTTGT AAGGTTTTGA TTTATCGT

48

<210> 1477  
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<220> bisulfite treated  
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<400> 1477  
GTTTCGTAAGG TTTTTCGGGA G

21

<210> 1478  
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<400> 1478  
TTCGGGCGAT TCGGTCGTTC GTGT

24

<210> 1479  
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<400> 1479  
CGAACCAACT CTAATCGCTC

20

<210> 1480  
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<400> 1480  
GAACCAACTC TAATCGCTCG

20

<210> 1481  
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<400> 1481  
TCGAACCAAC TCTAATCGCT

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<210> 1482  
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<400> 1482  
TCGAACCAAC TCTAATCGCT C

21

<210> 1483  
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<400> 1483  
ACGATCAATA ACAACCGAAC AC

22

<210> 1484  
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<400> 1484  
GCGTTTCGTT CGTAAGGTTT

20

<210> 1485  
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<400> 1485  
GCGTATTTTCG GAGAAGAAGT

20

<210> 1486  
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<400> 1486

TAGTCGTTTA GGAGAGTTTT TCG

23

<210> 1487

<211> 19

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1487

GATTCGGTCG TTCGTGTTC

19

<210> 1488

<211> 24

<212> DNA

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<400> 1488

AACACCGCGA CTATTATACG ACTA

24

<210> 1489

<211> 23

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1489

TTCGGTTGTT ATTGATCGTT TTA

23

<210> 1490

<211> 19

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1490

CTTCCGAACC CCGACTAAT

19

<210> 1491

<211> 20

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
<400> 1491  
AATTATTAGT CGGGGTTCGG

20

<210> 1492  
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<400> 1492  
AGCGCGTTCG GAAATCGAGG TTTTATTAAG T

31

<210> 1493  
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<400> 1493  
TACTCGACTC CGAAAACAAC TAA

23

<210> 1494  
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<400> 1494  
CCTCGAACCA ACTCTAATCG

20

<210> 1495  
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<400> 1495  
CGATCAATAA CAACCGAACA C

21

<210> 1496  
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 <400> 1496  
 GTTAGTTAGG TTCGTAGGCG TTA

23

<210> 1497  
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 <400> 1497  
 TCGTAAGGTT TTTCGGGAG

19

<210> 1498  
 <211> 21  
 <212> DNA  
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 <400> 1498  
 GGTAGTGCGA GCGATTAGAG T

21

<210> 1499  
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<220> bisulfite treated  
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 <400> 1499  
 TTCGTAAGGT TTTTCGGGAG

20

<210> 1500  
 <211> 19  
 <212> DNA  
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 <400> 1500  
 CGAGGTTTTT GTAGGTCGC

19

<210> 1501  
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<212> DNA  
<213> Artificial Sequence  
  
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<400> 1501  
TTTTTGTGTG TCGCGTTAAG TC

22

<210> 1502  
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<212> DNA  
<213> Artificial Sequence  
  
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<400> 1502  
GTTTCGGGTT GTAAAGTTTC G

21

<210> 1503  
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<212> DNA  
<213> Artificial Sequence  
  
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<400> 1503  
TTGGGTAAAG TCGCGTAGCG CGGT

24

<210> 1504  
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<212> DNA  
<213> Artificial Sequence  
  
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<400> 1504  
AGTAATTTTG TTTCGGGTCG T

21

<210> 1505  
<211> 21  
<212> DNA  
<213> Artificial Sequence  
  
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
<400> 1505  
GAACCTAACC GTAAAAACGC C

21

<210> 1506  
<211> 19  
<212> DNA  
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<220> bisulfite treated  
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<400> 1506  
CTACAAAAAC CTCGACCCG

19

<210> 1507  
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<212> DNA  
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<400> 1507  
GTTTCGTGTTT GGTGTTATT GA

22

<210> 1508  
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<212> DNA  
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<400> 1508  
ATTTTTATTTC CCCGCTAACG A

21

<210> 1509  
<211> 23  
<212> DNA  
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<220> bisulfite treated  
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<400> 1509  
ATTTTCGGTAA ATTCGTAGGT TTT

23

<210> 1510  
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<212> DNA  
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<220> bisulfite treated  
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<400> 1510  
ACCAACTACG TCGAACCAA

19

<210> 1511  
<211> 24  
<212> DNA  
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<220> bisulfite treated  
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<400> 1511  
GTTGGGTCGT AAGTTTTATA GTCG

24

<210> 1512  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220> bisulfite treated  
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<400> 1512  
TAATTTTGT TGTTCGCGTT AAG

23

<210> 1513  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220> bisulfite treated  
<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
<400> 1513  
CTCGACTCCG AAAACAATA AC

22

<210> 1514  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220> bisulfite treated  
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<400> 1514  
TCGGGAGTTA CGGTTTGA

18

<210> 1515  
<211> 22  
<212> DNA  
<213> Artificial Sequence

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<400> 1515  
GATAACGCCT ACGAACCTAA CT

22

<210> 1516  
<211> 19  
<212> DNA  
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
<400> 1516  
CGGGTCGAGG TTTTGTAG

19

<210> 1517  
<211> 37  
<212> DNA  
<213> Artificial Sequence

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<400> 1517  
GGTTTTGATT TATCGTTGTT TTTTCGTAA GTTCGGT

37

<210> 1518  
<211> 24  
<212> DNA  
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<400> 1518  
TTCGTCGTTT AGGAGAGTTT TTCG

24

<210> 1519  
<211> 19  
<212> DNA  
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
<400> 1519  
CGAAACCTAC TCGACTCCG

19

<210> 1520  
<211> 31  
<212> DNA  
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<400> 1520  
GATCGTTTTA ACGTCGTTAG GGACGATTCG T

31

<210> 1521  
<211> 20  
<212> DNA  
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<400> 1521  
TCGTGTTCGG TTGTTATTGA

20

<210> 1522  
<211> 19  
<212> DNA  
<213> Artificial Sequence

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<400> 1522  
CCAACCTACGT CGAACCAAA

19

<210> 1523  
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<212> DNA  
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<400> 1523  
TTTTTATTCC CCGCTAACGA

20

<210> 1524  
<211> 21  
<212> DNA  
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<400> 1524  
ACCTCGAACC AACTCTAATC G

21

<210> 1525  
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<212> DNA  
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
<400> 1525  
CGGTTTTTAG GGTGAGTCG

19

<210> 1526  
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<212> DNA  
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<400> 1526  
CGCGACCTAC AAAAACCTC

19

<210> 1527  
<211> 19  
<212> DNA  
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<400> 1527  
GCGACCTACA AAAACCTCG

19

<210> 1528  
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<400> 1528  
GAAACCTACT CGACTCCGAA A

21

<210> 1529  
<211> 19  
<212> DNA  
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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4  
<400> 1529  
CGTTCGTGTT CGGTTGTTA

19

<210> 1530  
<211> 31  
<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1530

ATCGTTTAA CGTCGTTAGG GACGATTCGT T

31

<210> 1531

<211> 23

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1531

AAACGAACGA CCAATAAAAA CAT

23

<210> 1532

<211> 22

<212> DNA

<213> Artificial Sequence

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<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1532

AACGATCAAT AACAAACCGAA CA

22

<210> 1533

<211> 24

<212> DNA

<213> Artificial Sequence

<220> bisulfite treated

<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1533

TACGACTATA AACTTACGA CCCA

24

<210> 1534

<211> 37

<212> DNA

<213> Artificial Sequence

<220> bisulfite treated

<223> nucleic acid for analysis of methylation status of SEQ ID NO: 4

<400> 1534

TTACGATGGC GGGGCGGCGG CGCGAATATA TAAAGGA

37

<210> 1535